AMERICAN METHANOL INSTITUTE

AR201-1304

telephone: 202 / 467-5050
Fax: 202 / 331-9055

July 6, 2001

Administrator
US Environmental Protection Agency
Attn: Chemical Right to Know Program
P. O. Box 1473
Merrifield, VA 22116

Dear Administrator:

The American Methanol Institute Testing Group (AMITG) has previously submitted the robust summary for methanol for the HPV Challenge Program, AR-201 on behalf of the consortium identified in our commitment letter dated March 18, 1999. As requested by your staff, enclosed please find a Test Plan for Methanol. As stated in the previously submitted robust summary, the enclosed document further supports the conclusion that no further testing is needed for methanol. The information submitted adequately addresses all SIDS endpoints in the HPV program and no data gaps exist.

Sincerely,

John E. Lynn
President & CEO

MR 49548

800 Connecticut Avenue, NW, Suite 620 • Washington, DC 20006 • E-mail: AMI@methanol.org • Internet: www.methanol.org

AR201-13104A

TEST PLAN FOR METHANOL

CAS 67-56-1

TEST PLAN JUSTIFICATION

SPONSORED BY

THE AMERICAN METHANOL INSTITUTE
TESTING GROUP

800 CONNECTICUT AVE. N.W.

SUITE 620

WASHINGTON, DC 2006

METHANOL TEST PLAN

ENDPOINT	INFORMATION AVAILABLE	ACCEPTABLE	TESTING NEEDED
PHYSICAL-CHEMICAL DATA			
Melting Point	YES	YES	NO
Boiling Point	YES	YES	NO
Vapor Pressure	YES	YES	NO
Partition Coefficient	YES	YES	NO
Water Solubility	YES	YES	NO
ENVIRONMENTAL FATE/ PATHWAYS			
Photodegradation	YES	YES	NO
Stability in Water	YES	YES	NO
Transport between Compartments	YES	YES	NO
Biodegradation	YES	YES	NO
ECOTOXICITY			
Acute Toxicity – Fish	YES	YES	NO
Toxicity – Aquatic Invertebrates	YES	YES	NO
Acute Toxicity – Aquatic Plants	YES	YES	NO
TOXICITY			
Acute Toxicity – Mammals	YES	YES	NO
Genetic Toxicity – in vivo	YES	YES	NO
Genetic Toxicity – in vitro	YES	YES	NO
Repeat Dose Toxicity	YES	YES	NO
Toxicity to Reproduction	YES	YES	NO
Developmental Toxicity/Teratogenicity	YES	YES	NO

INTRODUCTION

Methanol occurs naturally in plants and animals. It is a feedstock for chemical syntheses (for formaldehyde, acetic acid, and methyl tertiary-butyl ether) and a solvent in a variety of consumer products,

In humans, methanol is derived both from the diet and from metabolic processes (See robust summary). People ingest low doses of methanol in fruits, vegetables, and fermented beverages as well as indirectly from soft drinks and foods sweetened with aspartame (which breaks down to methanol in the gastrointestinal tract)

There is abundant data on the potential health effects of methanol in humans derived from clinical observations following accidental or intentional ingestion of methanol. Methanol can be highly toxic resulting in nausea, dizziness, metabolic acidosis, and toxicity to the visual system (including blindness), motor disturbances and even death in humans.

PHYSICAL-CHEMICAL DATA

Methanol is a widely used colorless, water-soluble simple alcohol containing one carbon atom. The physical-chemical properties are well known and found in standard references and texts. Original reports on which these citations are based are not available, but the information has been widely accepted based on many years of use. No further testing is need (See robust summary).

ENVIRONMENTAL FATE/PATHWAY

Alcohols generally do not hydrolyze in water. In a soil/water environment, methanol will be present primarily in the water phase. The dissolved methanol will migrate at near the velocity of groundwater except in soils with organic carbon fraction greater than 10 percent. Methanol in aqueous solution exhibited no degradation when exposed to sunlight using an EPA test protocol. Sediment and clay suspension solutions did not photocatalyze the degradation of methanol in aqueous solution during irradiation with UV light. The biodegradation of methanol has been studied under a wide variety of conditions and media, including wastewater, surface water, sediments, groundwater, and in soil microcosms. Methanol is completely degraded and there are no persistent degradation intermediates. No further testing is need (See robust summary).

ECOTOXICITY

A summary of the numerous reports of acute toxicity data shows LC50 values for fish range from 1,400 to 41,000 mg/l. Methanol is sometimes used as a carrier solvent in aquatic toxicology studies. Therefore, numerous chronic toxicity tests have, in fact, been conducted with methanol. For instance, both the USEPA TSCA fish bioconcentration test protocol (40 CFR 797.1560) and the ASTM standard guide for conducting early life-stage toxicity tests with fishes (ASTM E1241-92) specifically allow methanol as a carrier solvent at concentrations not to exceed 0.1 ml/L. Acute toxicity is directly related to the octanol-water partition coefficient; as log Pow increases, toxicity increases (e.g., LC50 decreases). Therefore, neutral compounds with low octanol-water partition coefficients, such as methanol, have very low acute toxicity. In invertebrates acute toxicity data for methanol shows a median effect concentrations (EC50 values) for immobilization range from 10,000 to 38,000 mg/L. Adverse effects (mortality, growth inhibition) occurred when methanol exposures to aquatic plants were in excess of 1,000 mg/L. No further testing is need (See robust summary).

TOXICITY

ACUTE

The acute oral toxicity (LD50) has been reported in rats, mice, monkeys, dogs, swine and rabbits. The 18 studies reported in the robust summary are usually old and details are lacking, but the results are consistent, The LD50 is greater than 5,000 mg/kg in all species tested The acute inhalation toxicity (LC50) has been reported in rats, mice and cats. The 10 studies reported in the robust summary are usually old and details are lacking, but the results are also consistent. The 4 hour LC50 in rats ranged from 64,000 -98,600 ppm. In mice the LC50 was 41,000 ppm and in cats the LC50 was 65,700 ppm. The dermal LD50 in rabbits is 15,840 mg/kg. Based on the large database of old studies and the similar response in various studies, no further testing is need for acute toxicity (See robust summary).

REPEAT DOSE TOXICITY

A majority of the repeat dose studies are inhalation in rats and monkeys. A study in rats and monkeys exposed up to 5,000 ppm, 6 hours/day, 5 days/week for 4 weeks resulted in nasal irritation in rats but not monkeys as the only treatment related effects at the highest dose. NEDO conducted a series of inhalation studies in rats (12 and 24 months), mice (12 and 18 months) and monkeys (2 1 days, 12 months and 30 months). The exposures were 20 plus hours a day, every day. The nearly continuous exposure did not allow much time for clearance, which would be normal in industrial or consumer exposure. The NOAEL in the rat and mouse studies was 100 ppm based on body weight and organ weight effects. No treatment related increase in cancer was observed. In the monkeys various effects were noted at similar doses.

There is also a 90 day gavage study conducted by the EPA in rats. Organ weight and enzyme effects were seen at the highest dose only (2,500 mg/kg). The NOAEL was 500 mg/kg. Methanol was also evaluated in a drinking water study in mice exposed for a lifetime at levels up to 0.899%. No treatment related effects were reported.

Methanol was also evaluated in a skin painting in mice exposed for a lifetime. No treatment related effects were reported. These numerous studies give a good evaluation of repeat exposure effects of methanol, and no further testing is need for repeat dose toxicity (See robust summary).

GENETOXICITY IN VITRO

There are numerous in vitro studies on methanol. They are generally negative and no further testing is need for genetic effects (See robust summary)

GENETOXICITY IN VIVO

There are micronucleus (oral) and cytogenetic assays (inhalation) studies conducted on methanol. They are negative and no further testing is need for genetic effects (See robust summary)

TOXICITY TO REPRODUCTION

Chronic methanol inhalation exposures to 1800 ppm for 2.5 hours per day for up to 1 year did not cause overt maternal toxicity in m. fascicularis females. The menstrual cycles and the ability of females to conceive and give birth to healthy live-born infants were also unaffected. Methanol exposures were associated with a reduction in the length of pregnancy (not dose dependent). No decrease in offspring birth size was observed.

In a two-generation study inhalation exposure had some slight treatment-related effects in rats exposed at 1,000 ppm, but no effects on reproductive performance was noted. Hormone changes were noted in other studies of reproductive effects, but no effects on reproduction were reported. No further testing is need for reproductive toxicity. (See robust summary).

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Pregnant rats exposed by inhalation to 20,000 ppm of methanol for 7 hours per day produced slight maternal toxicity and a significant increase in congenital malformations. A non-statistical increase in

malformation was also reported at 10,000 ppm. inhalation exposure for 20 hours per day caused maternal and fetal toxicity in rats exposed at 5,000 ppm. Methanol is not considered teratogenic in this study. 1,000 ppm was a NOAEL for both the dam and the fetus.

There are several developmental studies in mice, which appears to be more sensitive to methanol than the rats or monkeys. In key inhalation study in mice significant increases in the incidence of exencephaly and cleft palate were observed at 5,000 ppm and above, increased embryo/fetal death at 7,500 ppm and above (including an increasing incidence of full- litter resorptions), and reduced fetal weight at 10,000 ppm and above. A dose-related increase in cervical ribs or ossification sites lateral to the seventh cervical vertebra was significant at 2,000 ppm and above. No signs of maternal toxicity were noted.. The NOAEL for the developmental toxicity in this study was 1,000 ppm. Other special developmental studies in mice looked more closely at nutritional status and at critical stage of gestation to better understand the response in mice.

In a chronic methanol inhalation study in monkeys with daily exposure up to 1800 ppm for 2.5 hours daily for up to 1 year methanol did not cause overt maternal toxicity. The ability of females give birth to healthy live-born infants was also unaffected. Methanol exposures were associated with a reduction in the length of pregnancy (not dose dependent). No decrease in offspring birth size was observed. No further testing is need for developmental toxicity. (See robust summary).

SPECIES DIFFERENCES

The toxicity of methanol varies greatly between different species, toxicity depending on the ability to metabolize formate. In cases of slow metabolism of formate, fatal poisoning occurs as a result of metabolic acidosis and neuronal toxicity, whereas, in animals that readily metabolize formate, CNS depression (coma, respiratory failure, etc.) is usually seen. Sensitive primate species (humans and monkeys) develop increased blood formate concentrations following high level methanol exposure, while resistant rodents, rabbits and dogs do not.

The normal blood concentration of methanol in humans from endogenous sources is less than 0.5 mg/liter (0.02 mmol/litter), but dietary sources may increase blood methanol level. Generally, transient Central Nervous System (CNS) effects appear above blood methanol levels of 200 mg/liter (6 mmol/liter); ocular symptoms appear above 500 mg/liter (16 mmol/liter) and fatalities have occurred in untreated patients with initial methanol levels in the range of 1500-2000 mg/liter (47-62 mmol/liter).

Animal tests were done over the years to obtain predictive information. Investigation of methanol toxicity in animals is somewhat limited because normal rodents exposed to methanol do not display the metabolic acidosis and toxicity to the visual system that occurs in humans.

Incorporation of kinetic parameters and the fraction of inhaled methanol absorbed in humans and rodents into kinetic models predict that an 8-hour exposure to 5,000 ppm methanol will produce some very different results in different species. The blood methanol level in the mouse is 13-l 8 times higher and in the rat it is 5 times higher than humans theoretically exposed to the same 5,000 ppm inhaled level. This species difference may be related to the difference in response of pregnant animals to methanol. The mouse is the most sensitive showing developmental effects below a maternal toxic dose while the rat only response at higher doses that are maternal toxic.

There is abundant data on the potential health effects of methanol in animals and humans. Most information on the human health effects on methanol is derived from clinical observations following accidental or intentional ingestion of methanol. Based on the data in the robust summary no further testing is needed to complete the HPV data needs for methanol.

ROBUST SUMMARY OF TOXCITY OF METHANOL

INTRODUCTION

Methanol, a colorless, water-soluble simple alcohol containing one carbon atom, occurs naturally in plants and animals. Methanol has been used in industry for 100 years. It is a feedstock for chemical syntheses (for formaldehyde, acetic acid, and methyl tertiary-butyl ether and a solvent in a variety of consumer products (ie paints and varnishes, antifreeze, windshield washers, cleansing solutions, and adhesives) (World Health Organization 1997). Methanol is also a component or byproduct in various commercial operations such as sewage treatment, fermentation, and the pulp and paper industry.

In humans, methanol is derived both from the diet and from metabolic processes (Kavet and Nauss 1990, World Health Organization 1997). People ingest low doses of methanol in fruits, vegetables, and fermented beverages as well as soft drinks and foods sweetened with aspartame (which breaks down to methanol in the gastrointestinal tract)

There is abundant data on the potential health effects of methanol in humans. Most information on the human health effects of methanol is derived from clinical observations following accidental or intentional ingestion evrents. Methanol can be highly toxic resulting in nausea, dizziness, metabolic acidosis, and toxicity to the visual system (including blindness), motor disturbances and even death in humans. The absorption of methanol is rapid following oral ingestion, inhalation of methanol vapor, or skin contact. High doses of methanol overwhelm the body's ability to remove a toxic metabolite (formate). When formate accumulates, toxicity occurs

Animal tests were done over the years to obtain predictive information on health effects. Investigation of methanol toxicity in animals is somewhat difficult to correlate with human response because normal rodents (the most common laboratory test animal) exposed to methanol do not display the metabolic acidosis and toxicity to the visual system that occurs in humans (Roe 1982, Tephly and McMartin 1984; World Health Organization 1997).

Methanol is metabolized through the same pathways in humans and animals, but the differences in the rate of removal of metabolites result in the differences in methanol-induced toxicity. The data presented in this robust summary (IUCLID format) of key studies with some supplementary remarks and an added discussion about species differences supports the completeness of information needed for this HPV chemical.

This robust summary is prepared using the HPV subset of the IUCLID program format. The HPV subset calls for certain, not all, chapters of the IUCLID program. The chapters (profile) called for in the HPV program are listed on the next page. The use of the HPV subsets means the numbering of chapters is not always sequential. For some sections the IUCLID program uses drop down menus, while free text is used in other sections. The sections with drop down menu force a choice based on the drop down menu list. This can be confusing. For example, for question like what species was used might result in the choice of “other”(specific species not listed on drop down menu). In these cases we have indicate the specific species information in the method section which is a free text section.

I U C L I D

D a t a S e t

Existing Chemical	ID: 67-56-1
CAS No.	67-56-1
EINECS Name	methanol
EINECS No.	200-659-6
TSCA Name	Methanol
Molecular Formula	CH4O

Producer Related Part	
Company:	Bio Risk
Creation date:	22-FEB-2001
Substance Related Part	
Company:	Bio Risk
Creation date:	22-FEB-2001

Memo:	AMI

Printing date:	23-MAR-2001
Revision date:	24-FEB-2001
Date of last Update:	23-MAR-2001

Number of Pages:	65

Chapter (profile):	Chapter: 2.1, 2.2, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.1, 3.5, 4.1, 4.2, 4.3, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.8, 5.9
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

Date: 23-MAR-2001

2.1 Melting Point

Value: = -97.8 degree C
Decomposition: no
Sublimation: no
Method: other
Year: 1983
GLP: no data
Test substance: no data
Reliability: (2) valid with restriction
16-MAR-2001
(84)

2.2 Boiling Point

Value: = 64.6 degree C
Method: other
Year: 1990
GLP: no data
Test substance: no data
Reliability: (4) not assignable
16-MAR-2001
(96)

Value: = 64.7 degree C at 760 hPa
Decomposition: no
Method: other
Year: 1983
GLP: no data
Test substance: no data
Remark: no data
Reliability: (2) valid with restrictions
14-MAR-2001
(84)

2.4 Vapour Pressure

Value: = 127 hPa at 25 degree C
Decomposition: no
Method: other (measured)
Year: 1984
GLP: no data
Test substance: no data
Reliability: (2) valid with restriction
08-MAR-2001
(15)

2.5 Partition Coefficient

log Pow: = -.77
Method: other (calculated)
Year: 1996
GLP: no data
Test substance: no data
Remark: See Sanger J., Octanol-water partition of simple organic compounds (1989) J. Phys. Chem. Ref. Data, Vol 18 No.3 p1150. Sanger reports a range of Log P from -0.32 to 0.83.
Reliability: (2) valid with restrictions
08-MAR-2001
(36)

2.6.1 Water Solubility

Qualitative: miscible
pKa: 15 at 25 degree C
pH: = 7
Method: other
Year: 1985
GLP: no data
Test substance: no data
Reliability: (2) valid with restrictions
22-MAR-2001
(34)

3.1.1 Photodegradation

Type: air
DIRECT PHOTOLYSIS
Degradation: = 50 % after 17.8 day
INDIRECT PHOTOLYSIS
Sensitizer: OH
Conc. of sens.: 500000 molecule/cm3
Method: other (calculated)
Year: 1990 GLP: no data
Test substance: no data
Remark: rate constant= 0.9x10-12 cm3/molecule sec
Reliability: (2) valid with restrictions
16-MAR-2001
(7)

Type: air
Method: other (measured)
Year: 1989 GLP: no data
Test substance: no data
Remark: rate constant= 0.88 (+/-) *10^-12 cm3/molecule*sec bei
Reliability: (2) valid with restrictions
16-MAR-2001
(6)

Type: water
INDIRECT PHOTOLYSIS
Sensitizer: OH
Method: other (calculated)
Year: 1991 GLP: no data
Test substance: no data
Deg. Products
Remark: k(OH)=8.46X10e-9 liter/mole OH concentration 5*10e-14 mol/liter, temperature 298K
Reliability: (2) valid with restrictions
16-MAR-2001
(46)

3.1.1 Photodegradation (Added Remarks)

Remark: Degradation of methanol by photolysis is not expected to be significant
Reliability: (2) valid with restrictions
21-MAR-2001
(31)

Remark: Methanol in aqueous solution exhibited no degradation when

exposed to sunlight using an EPA test protocol. Sediment and clay suspension solutions did not photocatalyze the degradation of methanol in aqueous solution during irradiation with uv light
Reliability: (2) valid with restrictions
21-MAR-2001
(37)

3.1.2 Stability in Water

Type:
Method: other
Year: 1982 GLP: no data
Test substance: no data
Remark: Alcohols generally do not hydrolyze in water
Reliability: (2) valid with restrictions
16-MAR-2001
(52)

Type:
Method:
Year: GLP:
Test substance:
Remark: Abiotic degradation (i.e., non-biological or chemical) reactions are not likely to contribute significantly to methanol removal from surface water bodies. Hydrolysis reactions usually transform compounds into more polar products; methanol is a very polar molecule and is stable in water. Methanol has a measured Henry's Law Constant of 4.4 X 10-6 atm-cu m/mole at 250C. This value of Henry's Law constant indicates that volatilization from environmental waters may be significant. The volatilization half-life from a river (1 meter deep flowing 1 M/sec with a wind speed of 3 m/sec) has been 4.8 days. Degradation (i.e., non-biological or chemical)reactions are not likely to contribute significantly to methanol removal from surface water bodies. Hydrolysis reactions usually transform compounds into more polar products; methanol is a very polar molecule and is stable in water. Methanol has a measured Henry's Law Constant of 4.4 X 10-6 atm-cu m/mole at 250C. This value of Henry's Law Constant indicates that volatilization from environmental waters may be significant. The volatilization half-life from a river (1 meter deep flowing 1 M/sec with a wind speed of 3 m/sec) has been 4.8 days.
Reliability: (2) valid with restrictions
22-MAR-2001
(61)

3.3.1 Transport between Environmental Compartments

Type: volatility
Media: water - air
Air (Level I):
Water (Level I):
Soil (Level I):
Biota (L.II/III):
Soil (L.II/III):

Method: other
Year: 1982
Remark: Methanol has a measured Henry's Law constant of 4.4 *10^-6 atm*m*3/mol at 25 deg C. Volatilization to environment maybe significant.
Reliability: (2) valid with restrictions
16-MAR-2001
(51)

Type: volatility
Media: water - air
Air (Level I):
Water (Level I):
Soil (Level I):
Biota (L.II/III):
Soil (L.II/III):
Method: other
Year: 1990
Remark: Howard estimated a half-life of 2.6 days for volatilization Of methanol from a pound. Using Henry's law constant and equation from Lyman (1982) half-life can be calculated for different conditions (wind, water flow rate and depth of water)..
Reliability: (2) valid with restrictions
16-MAR-2001
(40)

3.3.1 Transport between Environmental Compartments (Added Remarks)

Remark: The soil/water partition coefficient, Kd, can be used to estimate the rate of movement of a chemical in groundwater compared to the rate of groundwater flow. For non-ionic organic compounds such as methanol, Kd values are a function of the organic carbon content of the soil (foc) and the organic carbon based partition coefficient (Koc [L/kg]). In a soil/water environment, methanol will be present primarily in the water phase. The dissolved methanol will migrate at the velocity of groundwater except in soils with organic carbon fraction greater than 10 percent (i.e., for foc = 0.1 the Kd is approximately 0.8 signifying nearly equivalent concentrations of methanol adsorbed on soil and dissolved in water)
Reliability: (2) valid with restrictions
21-MAR-2001
(61)

Remark: Methanol is miscible and should have high mobility in soil and migrate with any surface water owing to low Ko/w value of -0.77. Based on a vapor pressure of 92mm Hg at 20 deg C evaporation form dry surfaces can be expected.
Reliability: (2) valid with restrictions
21-MAR-2001
(37)

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, adapted
Degradation: = 50 - 80 % after 6 day
Result: readily biodegradable
Method: other
Year: 1978 GLP: no data
Test substance: no data
Result: Adaptation of the sludge to 0.1% (v/v) methanol occurs over a period of several days. More than 80% of the methanol is then metabolized.
Reliability: (2) valid with restrictions
16-MAR-2001
(83)

Type: aerobic
Inoculum: activated sludge, adapted
1.5 g/l related to COD (Chemical Oxygen Demand)
Contact time: 29 day
Degradation: = 95 % after 20 day
Result: readily biodegradable
5 day = 76 %
10 day = 88 %
15 day = 91 %
20 day = 95 %
Deg. Product: not measured
Method: other
Year: 1974 GLP: no data
Test substance: no data
Remark: The biodegradation in seawater was 69% *5 days), 84% (10 days). 85% (15 days), and 97% (20 days).
Conclusion: Methanol is rapidly biodegraded in fresh and sea water.
Reliability: (2) valid with restrictions
16-MAR-2001
(63)

Type: anaerobic
Inoculum: predominantly domestic sewage
Contact time: 182 hour(s)
Degradation: >= 50 % after 7 hour(s)
Result: readily biodegradable
Deg. Product: yes
Method: other
Year: 1993 GLP: no data
Test substance: no data
Method: Sediment and groundwater were collected from a methaanogenic portion of a shallow anoxic aquifer polluted by municipal landfill leachate. Slurries were prepared by placing 50 g of sediment and 75 ml of groundwater in sterile 160-mL serum bottles. The bottles were sealed and incubated in the dark at room temperature. Each compound was added to the ncubation mixtures to reach an initial substrate concentration of 50 ppm. Pressure increases resulting from biogas formation (CH4 and C02) were monitored with an automated pressure transducer

system. At the end of the incubation period, the depletion of the parent substrate and the formation of methane over background controls were confirmed with a Varian 3300 gas chromatograph equipped with a flame ionization detector. The rate of substrate depletion was determined in incubations receiving a subsequent addition of the oxygenate. The amount of methane formed in aquifer incubations was compared to that theoretically expected based on the Buswell equation.

Conclusion: Methanol is rapidly biodegraded under anaerobic condition by sediment and groundwater polluted by municipal landfill leachate

Reliability: (2) valid with restrictions

16-MAR-2001

(82)

Type: anaerobic
Inoculum: other
Contact time: 4 month
Degradation: >= 98 % after 4 month
Result: readily biodegradable
Deg. Product: not measured
Method: other
Year: 1996 GLP: no data
Test substance: no data

Method: Microcosms were constructed in 0.5 or 1 liter capped glass bottles. The 1 liter bottles contained 200 grams of site soil and 0.9 liter of ground water. The 0.5 liter bottle had 150 grams of soil and 0,4 liter of water. Dissolved oxgen was measued. Hydrogen peroxide was added to some bottles ad an oxygen source.

Result: Methanol is degraded under conditions of this study.

Reliability: (2) valid with restrictions

16-MAR-2001

(64)

3.5 Biodegradation (Added Remarks)

Remark: The biodegradation of methanol has been studied under a wide variety of conditions and media, including wastewater, surface water, sediments, groundwater, and in soil microcosms. Methanol is completely degraded and there are no persistent degradation intermediates.

21-MAR-2001

(38)

Remark: Biodegradation is the predominant removal process for Methanol in activated sludge treatment of pulp and paper Industry wastewater.

21-MAR-2001

(8)

Remark: Methanol added to natural microbial assemblages taken from a eutrophic lake in Georgia, from the Okefenokee Swamp and from mangrove stands degraded with half-lives between nine and 29

days

21-MAR-2001
(41)

Remark: Half-life can be long in dry soils (weeks to months).

21-MAR-2001
(2)

Remark: Removal during biological wastewater treatment is reported at 86-99 percent. Aqueous half-lives for aerobic and anaerobic biodegradation of methanol ranging from 24 to 168 hours (7 days. Methanol estimated half-life range from one to seven days in saturated soils.

21-MAR-2001
(39)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: flow through
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: c = 15400
Method: other
Year: 1986 GLP: no data
Test substance: other TS
Method: Analytical values determined by spectrofluorimeter. Water obtained from Lake Superior. Water chemistry evaluated along with dissolved oxygen. Five concentrations tested.
Result: Effects were noted almost immediately at two highest concentration. Most of the mortality was noted in 3 hours.
Test substance: Methanol (Burdick & Jackson)
Reliability: (1) valid without restrictions
09-MAR-2001
(62)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: c = 28100
Method: other
Year: 1983 GLP: no data
Test substance: no data
Method: Five concentrations tested. Water chemistry determined. Water from Lake Superior. Analytical determination by spectrofluorimetry.
Remark: Call, D.J., L.T., Brooks, N., Ahamd, and J.E., Richter,
(1983) Toxicity and metabolism studies with EPA priority pollutants and related chemicals in freshwater organisms, EPA-600/3-83-095, PB83-263665 also report LC50 of 28100 mg/l
Reliability: (2) valid with restrictions
22-MAR-2001
(87)

Type: flow through
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: c = 29400
Method: other
Year: 1986 GLP: no data
Test substance: other TS
Method: Five concentrations tested. Water from Lake Superior. Water chemistry and dissolved oxygen levels determined.
Result: Effects noted at top two concentrations. Most mortality was noted in first 12 hours.
Test substance: Methanol (Burdick & Jackson)
Reliability: (1) valid without restriction
09-MAR-2001
(62)

Type: flow through
Species: Salmo gairdneri (Fish, estuary, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: yes
LC50: c = 20300
Method: other
Year: 1985 GLP: no data
Test substance: other TS
Method: Five concentrations used. Analytical determination by spectrofluorimeter. Water from Lake Superior. Water chemistry determined as well as dissolved oxygen. Trimed Spearman Karber statistical method used.
Result: Effects were noted immediately at two highest concentrations. Most mortality was noted by 3 hours.
Test substance: Methanol (Burdick & Jackson)
Reliability: (1) valid without restrictions
09-MAR-2001
(62)

Type: semistatic
Species: Oryzias latipes (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: c <= 5000 - 5000
LC50: m = 35000 - 28000
LC100: c >= 40000 - 40000
Method: other
Year: 1985 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: 8 levels 0-40,000 ppm. Measurements at 24, 48 72 and 96 hours. Avoidance and embrogensis (12 day exposure) also evaluated.
Remark: Method similar to OECD,
Result: The first results are 96-hour values, the second are 24 hour values. Avoidance was demonstrated at 10,000-20,000 ppm. Effective half lethal dose on embryogensis -20,000 ppm, tolerance dose - 1000 ppm, Critical toxic dose 30,000 ppm.
Reliability: (2) valid with restrictions
09-MAR-2001
(58)

Type: semistatic
Species: other
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: c = 4000 - 4000
LC50: c = 12000 - 13000
LC100: c = 15000 - 20000
Method: other
Year: 1985 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: Red sea bream (Pagrus major) was the test organism used in this test. Eleven test levels were used (0-40,000 ppm).

Measurements were also conducted at 24, 48 and 72 hours
Remark: Limited data. Method similar to OECD,
Reliability: (2) valid with restrictions
16-MAR-2001
(58)

Type: semistatic
Species: other
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: c = 4000 - 4000
LC50: c = 12000 - 13000
LC100: c = 15000 - 20000
Method: other
Year: 1985 GLP: no data
Test substance: other TS
Method: Red sea bream (Pagrus major) used in test. Eleven levels (0-40000) used. Measurements also conducted at 24, 48 and 72 hours. Avoidance testing also conducted.
Result: First value is the 96-hour value, the second is the 24 hour value. Avoidance was demonstrated between 10,000 - 20,000 ppm.
Test substance: Methanol, Junsei Chemical Co.
Reliability: (2) valid with restrictions
09-MAR-2001
(58)

Type: semistatic
Species: other
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: c = 5000 - 1200
LC50: c = 13000 - 25000
LC100: c = 20000 - 40000
Method: other
Year: 1985 GLP: no data
Test substance: other TS
Method: Tiger shrimp (Penaeus japonicus) used in test. Eight levels were used (0-40000 ppm). Test design similar to OECD. Measurements also at 24, 48 and 72 hours. Avoidance testing also conducted.
Result: First value is 96-hour value, the second value is 24 hour value. Avoidance demonstrated at 5000 ppm
Test substance: Methanol, Junsei Chemical Co.
Reliability: (2) valid with restrictions
09-MAR-2001
(58)

Type: semistatic
Species: other
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC0: c = 2000 - 10000

LC50: c = 15000 - 30000
LC100: c >= 40000 - 40000
Method: other
Year: 1985 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: 8 levels 0-40,000 ppm. Measurements at 24, 48, 72, and 96 hours. Ear shell (Haliortis discus hanni) which is a young shell fish was used.
Remark: Method was similar to OECD
Result: First value is the 96-hour value, the second is 24-hour value.
Reliability: (2) valid with restrictions
22-MAR-2001
(58)

Type: static
Species: Alburnus alburnus (Fish, estuary)
Exposure period: 96
Unit: mg/l Analytical monitoring: no data
LC50: c = 28000
Method: other
Year: 1984 GLP: no data
Test substance: no data
Method: A natural brackish water from Baltic sea was used. Water Chemistry was determined, 2 replicates, 10 fish per replicate. Number of concentrations tested unknown.
Reliability: (2) valid with restrictions
22-MAR-2001
(12)

Type: static
Species: Pimephales promelas (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: c > 100
Method: other
Year: 1985 GLP: no data
Test substance: no data
Method: Water from Lake Ontario. Test concentrations used 0, 1, 10 And 100 mg/l. Ten fish per replicate. Also tested pillibugs, daphnia magna, flatworms, side swimmers and the segment worm at same concentrations.
Remark: A screening test with 100 mg/l the highest concentration tested.
Result: The 96 hour LC50 was greater than 100 mg/l for all test organisms.
Reliability: (2) valid with restrictions
09-MAR-2001
(33)

Type: static
Species: other
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no data

LC50: c = 12000
Method: other
Year: 1984 GLP: no data
Test substance: no data
Method: Nitocra spinipes used as test organism. Water chemistry determined. Water use was natural brackish water from the Baltic sea. Number of concentration tested unknown.
Reliability: (1) valid without restriction
09-MAR-2001
(12)

Type: other
Species: Lepomis macrochirus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no
LC50: c = 29.4
Method: other
Year: 1985 GLP: no data
Test substance: no data
Reliability: (2) valid with restrictions
14-MAR-2001
(50)

4.1 Acute/Prolonged Toxicity to Fish (Added Remarks)

Remark: A summary of acute toxicity data (Appendix B Table B-1) shows a range of LC50 values for fish range from 1,400 to 41,000 mg/l. A QSAR calculation of the a 96 hour LC50 for salt water fish gives a value of 572 mg/l, while the value for freshwater fish and Mysid shrimp is > 1000 mg/l
21-MAR-2001
(31)

Remark: Methanol is sometimes used as a carrier solvent in aquatic toxicology studies. Therefore, numerous chronic toxicity tests have, in fact, been conducted with methanol. For instance, both the USEPA TSCA fish bioconcentration test protocol (40 CFR 797.1560) and the ASTM standard guide for conducting early life-stage toxicity tests with fishes (ASTM E1241-92) specifically allow methanol as a carrier solvent at concentrations not to exceed 0. 1 ml/L (100 mg/L).
21-MAR-2001

Remark: Acute toxicity is directly related to the octanol-water partition coefficient; as log OW increases, toxicity increases (e.g., LC50 decreases). Therefore, neutral compounds with low octanol-water partition coefficients, such as methanol, have very low acute toxicity. Acute toxicity via narcosis is generally reversible. In fish, narcosis produces a specific series of behavioral stages including loss of reaction to external stimuli; loss of equilibrium; a decline in respiratory rate; and finally,

21-MAR-2001
(72)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Artemia salina (Crustacea)
Exposure period: 24 hour(s)
Unit: mg/l Analytical monitoring: no data
EC0: c > 10000
Method: other
Year: 1974 GLP: no
Test substance: no data
Method: Concentrations tested (100, 1,000 and 10,000 mg/l). Endpoint evaluated was death.
Reliability: (1) valid without restriction
09-MAR-2001
(63)

Type: static
Species: Ceriodaphnia dubia (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no
EC50: c = 11
Method: other
Year: 1993 GLP: no data
Test substance: no data
Remark: The LC50 (48 hrs) for freshwater mussel (*Anadonta imbecilis)* was also determined
in this study (37.02 mg/l).
Reliability: (1) valid without restriction
14-MAR-2001
(44)

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l Analytical monitoring: no data
EC0: c > 10000
Method: other
Year: 1988 GLP: no data
Test substance: no data
Method: Method DIN 38412. Concentrations tested (0-10000 ppm). Immobilization endpoint evaluated. Water chemistry determined.
Remark: For similar study see Calleja, M.C., G. Persoone, and P.Geadi, (1994), Comparative acute toxicity of the first 50 multicentre evaluations of in vitro cytotoxicity chemicals to aquatic non-vertebrates. Arch. Environ. Contam. Toxicol. 26, 69 - 78 The L(E)C50 for methanol in Daphnia Magna was reported as 668,000 umol/l).
Reliability: (1) valid without restriction
09-MAR-2001
(47)

Type: static
Species: Nitocra spinipes (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l Analytical monitoring: no data
EC50: c = 12000
Method: other
Year: 1984 GLP: no data
Test substance: no data
Method: A natural brackish water from Baltic sea was used. Water chemistry was determined.
Reliability: (1) valid without restrictions
09-MAR-2001
(12)

4.2 Acute Toxicity to Aquatic Invertebrates (Added Remarks)

Remark: Acute toxicity data for methanol in invertebrates were summarized in an Environ Corporation (1996)report in appendix B, table B-2. The measured LC50 values and median effect concentrations (EC50 values) for immobilization in invertebrate range from 10,000 to 38,000 mg/L. The calculated QSAR 48 hour LC50 is greater than 1000 mg/l.
21-MAR-2001
(32)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: Chlorella pyrenoidosa (Algae)
Endpoint: growth rate
Exposure period: 14 day
Unit: mg/l Analytical monitoring: no
NOEC: c = 4% v/v
LOEC: c < 4% v/v
EC50: m < 3.6% v/v = 28.44 g/l
Method: other
Year: 1988 GLP: no data
Test substance: no data
Reliability: (2) valid with restrictions
13-MAR-2001
(81)

Species: Phaeodactylum tricornutum (Algae)
Endpoint: other
Exposure period: 840 hour(s)
Unit: mg/l Analytical monitoring: no
NOEC: c > 100
LOEC: c > 100
EC50: c = 600
Method: OECD Guideline 201 "Algae, Growth Inhibition Test"
Year: 1985 GLP: no

Test substance: no data
Reliability: (1) valid without restrictions
13-MAR-2001
(57)

Species: Phaeodactylum tricornutum (Algae)
Endpoint: other
Exposure period: 35 day
Unit: mg/l Analytical monitoring: no data
LOEC: c 1000
EC50: m = 600
Method: other
Year: 1985 GLP: no data
Test substance: no data
Remark: Study was conducted to evaluate the effect of methanol on alga reproduction according to report
Reliability: (2) valid with restrictions
08-MAR-2001
(56)

4.3 Toxicity to Aquatic Plants e.g. Algae (Added Remarks)

Remark: A summary table of acute toxicity data for methanol in Aquatic plants is found in Appendix B, Tables B-3 of the Environ Corporation (1996) report. Adverse effects mortality, growth inhibition)occurred when methanol exposures were in excess of 1,000 mg/L. The calculated QSAR 96 hour EC50 for green algae was greater than 1000 mg/l
21-MAR-2001
(32)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain:
Sex:
Number of Animals:
Vehicle:
Value: = 5628 mg/kg bw
Method: other
Year: 1994 GLP: no data
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the

results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(67)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 9100 mg/kg bw
Method: other
Year: 1943 GLP: no
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(91)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 9540 mg/kg bw
Method: other
Year: 1972 GLP: no
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(90)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 11520 mg/kg bw
Method: other
Year: GLP: no data
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.

Reliability: (2) valid with restrictions
21-MAR-2001
(85)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 12750 mg/kg bw
Method: other
Year: 1967 GLP: no
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(25)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 12880 mg/kg bw
Method: other
Year: 1941 GLP: no
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (4) not assignable
21-MAR-2001
(77)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 5065 mg/kg bw
Method: other
Year: 1961 GLP: no
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.

Reliability: (2) valid with restrictions
21-MAR-2001
(9)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 7914 mg/kg bw
Method: other
Year: 1975 GLP: no data
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(10)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 12900 mg/kg bw
Method: other
Year: 1948 GLP: no
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(28)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 10300 mg/kg bw
Method: other
Year: 1971 GLP: no data
Test substance: no data
Remark: LD50 listed is for young adult rat. LD50 for 14 day old rat = 5861 mg/kg bw and 6970 mg/kg bw for old adult rat.

Reliability: (2) valid with restrictions
12-MAR-2001
(45)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 7300 mg/kg bw
Method: other
Year: 1994 GLP: no data
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(67)

Type: LD50
Species: mouse
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 5800 mg/kg bw
Method: other
Year: 1969 GLP: no
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(20)

Type: LD50
Species: mouse
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 14390 mg/kg bw
Method: other
Year: 1971 GLP: no data
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.

Reliability: (2) valid with restrictions
21-MAR-2001
(76)

Type: LD50
Species: rabbit
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 14400 mg/kg bw
Method: other
Year: 1972 GLP: no data
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(54)

Type: LD50
Species: dog
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 8000 mg/kg bw
Method: other
Year: 1997 GLP: no data
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(93)

Type: LD50
Species: dog
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: = 7500 mg/kg bw
Method: other
Year: 1994 GLP: no data
Test substance: no data
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Reliability: (2) valid with restrictions

21-MAR-2001
(67)

Type: LD50
Species: miniature swine
Strain: other
Sex: female
Number of Animals: 3
Vehicle: no data
Value: > 5000 mg/kg bw
Method: other
Year: 1993 GLP: no data
Test substance: other TS
Remark: Details of the study maybe lacking in some areas, but the results are similar in all studies.
Test substance: Methanol - HPLC grade Sigma Chemical
Reliability: (1) valid without restriction
21-MAR-2001
(29)

Type: LD50
Species: monkey
Strain:
Sex:
Number of Animals:
Vehicle:
Value: = 7000 - 9000 mg/kg bw
Method: other
Year: 1961 GLP: no
Test substance: no data
Remark: Monkeys receiving methanol doses higher than 3000 mg/kg by gavage show ataxia, weakness and lethargy within a few hours of exposure These signs tended to disappear within 24 hours and were followed by transient coma in some of the animals. Details are lacking in some areas, but are similar to other studies.
Reliability: (2) valid with restrictions
21-MAR-2001
(23)

5.1.2 Acute Inhalation Toxicity

Type: LC50
Species: rat
Strain:
Sex:
Number of Animals:
Vehicle:
Exposure time: 1 hour(s)

Value: = 145000 ppm
Method: other
Year: GLP: no data
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Test condition: Exposure was head only.
Reliability: (2) valid with restrictions
22-MAR-2001
(30)

Type: LC50
Species: rat
Strain:
Sex:
Number of Animals:
Vehicle:
Exposure time: 4 hour(s)
Value: = 64000 ppm
Method: other
Year: 1994 GLP: no data
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Reliability: (2) valid with restrictions
22-MAR-2001
(67)

Type: LC50
Species: rat
Strain:
Sex:
Number of Animals:
Vehicle:
Exposure time: 4 hour(s)
Value: = 73000 ppm
Method: other
Year: 1982 GLP: no data
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Reliability: (2) valid with restrictions
22-MAR-2001
(76)

Type: LC50
Species: rat
Strain:
Sex:
Number of Animals:

Vehicle:
Exposure time: 4 hour(s)
Value: = 98600 ppm
Method: other
Year: 1980 GLP: no data
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Reliability: (2) valid with restrictions
14-MAR-2001
(11)

Type: LC50
Species: rat
Strain:
Sex:
Number of Animals:
Vehicle:
Exposure time: 6 hour(s)
Value: = 67300 ppm
Method: other
Year: 1980 GLP: no data
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Reliability: (2) valid with restrictions
22-MAR-2001
(11)

Type: other
Species: rat
Strain:
Sex:
Number of Animals:
Vehicle:
Exposure time: 8 hour(s)
Value: = 64000 ppm
Method: other
Year: GLP: no
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Test condition: Most likely saturated vapor exposure.
Reliability: (2) valid with restrictions
22-MAR-2001
(78)

Type: LC50
Species: mouse
Strain:
Sex:
Number of

Animals:
Vehicle:
Exposure time: 6 hour(s)
Value: = 41000 ppm
Method: other
Year: 1979 GLP: no data
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Reliability: (2) valid with restrictions
22-MAR-2001
(73)

Type: LC50
Species: mouse
Strain:
Sex:
Number of
Animals:
Vehicle:
Exposure time: 134 minute(s)
Value: = 61100 ppm
Method: other
Year: 1994 GLP: no data
Test substance: no data
Remark: Details are lacking in some areas, but the rfesult is similar in all studies.
Reliability: (2) valid with restrictions
14-MAR-2001
(88)

Type: LC50
Species: cat
Strain:
Sex:
Number of
Animals:
Vehicle:
Exposure time: 4.5 hour(s)
Value: = 65700 ppm
Method: other
Year: 1994 GLP: no data
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Reliability: (2) valid with restrictions
22-MAR-2001
(88)

Type: LC50
Species: cat
Strain:
Sex:

Number of
  Animals:
Vehicle:
Exposure time: 6 hour(s)
Value: = 23600 ppm
Method: other
  Year: 1994 GLP: no data
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Reliability: (2) valid with restrictions
22-MAR-2001
(88)

Type: LC50
Species: cat
Strain:
Sex:
Number of
  Animals:
Vehicle:
Exposure time:
Value: = 20000 - 36900 ppm
Method: other
  Year: 1931 GLP: no
Test substance: no data
Remark: Details are lacking in some areas, but the result is similar in all studies.
Reliability: (2) valid with restrictions
22-MAR-2001
(94)

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain:
Sex:
Number of
  Animals:
Vehicle:
Value: = 15840 mg/kg bw
Method: other
  Year: 1994 GLP: no data
Test substance: no data
Reliability: (2) valid with restrictions
12-MAR-2001
(67)

Type:	other
Species:	rabbit
Strain:	
Sex:	
Number of Animals:	
Vehicle:	
Value:	
Method:	
Year:	GLP:
Test substance:	
Remark:	In a study in rabbits conducted according to OECD guideline 404 methanol was classified as non-irritating to the skin, but an irritant to the eye (OECD guideline 405).
Reliability:	(1) valid without restrictions

22-MAR-2001
(43)

5.4 Repeated Dose Toxicity

Species:	rat	Sex:	male/female
Strain:	Sprague-Dawley		
Route of admin.:	inhalation		
Exposure period:	6 hours/day		
Frequency of treatment:	5 days/week for 4 weeks		
Post. obs. period:	none		
Doses:	0, 500, 2000, 5000 ppm		
Control Group:	yes, concurrent vehicle		
NOAEL:	>= 3000 ppm		
LOAEL:	= 5000 ppm		
Method:	other		
Year:	1987	GLP:	yes

Test substance: other TS
Method: The study used 5 rats /sex/ group. Parameters evaluated included, ophthalmoscopic exam, body weight, clinical signs, organ weights, histopathology and survival
Remark: A good screening study. Daily exposure was 6 hours per day, the normal daily exposure length for inhalation studies evaluating potential workplace exposure.
Result: Actual exposure 0, 520, 1980 and 5010 ppm. All animals survived. In rats nasal and eye discharge (mucoid, nasal, red nasal, lacrimation) was noted in the treatment groups. Only mucoid nasal discharge appeared to be dose related. There was no treatment-related effects on body weight. No ocular abnormalities were noted at the terminal ophthalmoscopic exam. No treatment related histopathological effects (35 tissues)were noted, but spleen weights were increased in female rats exposed at 2,000 ppm (not at 5,000 ppm). No other organ weight effects were noted.
Test substance: Methanol from Celanese Corporation 99.85% pure
Conclusion: Rats were exposed to up to 5,000 ppm (6 hr/d, 5d/wk for 4 weeks) showed no treatment related histopathological effects. Inhalation exposure resulted in some slight treatment-related signs of nasal irritation in rats exposed at 5,000 ppm. No effects were noted in the ophthalmoscopic exam. Overall the results support the use of the present TLV for methanol
Reliability: (1) valid without restriction
14-MAR-2001
(4)

Species: rat Sex: male/female
Strain: Fischer 344
Route of admin.: inhalation
Exposure period: 20 hours/day
Frequency of treatment: daily for 1 year
Post. obs. period: none
Doses: 0, 10, 100, 1000 ppm
Control Group: yes
NOAEL: >= 100 ppm
LOAEL: = 1000 ppm
Method: other
Year: 1986 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: This study used 20/sex per group. Parameters evaluated body weight, food consumption, clinical signs, hematology, organ weight, histopathology, clinical chemistry, and survival.
Remark: This chronic inhalation study design was similar to standard chronic studies in rats except the length of the daily exposure was much longer than normal (20 vs 6 hrs). The design offered little time for normal clearance from the body as exposure was essentially continuous (not normal for industrial exposure or consumer use).
Result: In both males and females exposed at 1,000 ppm a slight decrease in body weight gain was noted at the end of 12

months. Rats exposed at all levels showed no clinical signs or clinical chemistry effects, but increase in liver and spleen weights was noted in female rats (less than 5%). At 100 ppm and lower, no pathological changes due to treatment were noted. One rat died and one was sacrificed during the study (exposure level not indicated). No treatment related effects were reported for food consumption, hematology, antibody test, urinalysis, serum or biochemical tests.

Conclusion: The NOAEL in rats appears to be 100 ppm with only small body weight changes and possible organ weights the only effects seen at in rats exposed at 1,000 ppm

Reliability: (2) valid with restrictions

14-MAR-2001

(58)

Species: rat Sex: male/female

Strain: Fischer 344

Route of admin.: inhalation

Exposure period: 20 hours/day

Frequency of treatment: daily for two years

Post. obs. period: none

Doses: 0, 10, 100, 1000 ppm

Control Group: yes

NOAEL: > 100 ppm

LOAEL: <= 1000 ppm

Method: other

Year: 1986 GLP: no data

Test substance: Methanol, Junsei Chemical Co.

Method: This study used 52/sex per group. Parameters evaluated body weight, food consumption, clinical signs, hematology, organ weight, histopathology, clinical chemistry, and survival.

Remark: This carcinogenicity study design was similar to standard carcinogenicity studies in rats except the length of the daily exposure was much longer than normal (20 vs 6 hrs. The design offered little time for normal clearance from the body as exposure was essentially continuous (not normal for industrial exposure or consumer use).

Result: No treatment related effects were reported for clinical signs, body weight, organ weight food consumption, hematology, antibody test, urinalysis, serum or biochemical tests. A small increase in papillary adenoma of the lung as well as an increase in adenomatosis were the only histopathological changes noted in the high dose males. In the high dose females the incidence of chromaffine cytoma of the adrenal gland was low but slightly higher than the control.

Conclusion: Although a possible small increase in benign tumors were observed in the high dose animals, methanol is not considered carcinogenic based on the results of this study.

Reliability: (2) valid with restrictions

14-MAR-2001

(58)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of admin.: inhalation
Exposure period: 20 hours/day
Frequency of treatment: daily for 18 months
Post. obs. period: none
Doses: 0, 10, 100, 1000 ppm
Control Group:
NOAEL: > 1000 ppm
Method: other
Year: 1986 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: Fifty-two per sex per group were studied. Parameters evaluated include clinical signs, organ weight, body weight, food consumption, hematology, urinalysis, histopathology and serum or biochemical tests.
Remark: This carcinogenicity study design was similar to standard carcinogenicity studies in mice except the length of the daily exposure was much longer than normal (20 vs 6 hrs). The lack of individual data is limitation of this study. The design offered little time for normal clearance from the body as exposure was essentially continuous (not normal for industrial exposure or consumer use).
Result: No treatment related effects were reported for clinical signs, organ weight, food consumption, hematology, urinalysis, and serum or biochemical tests. Body weight was significantly higher in the high dose group early in the study, but was not different by 12 months. No tumorgenic effects were treatment related.
Conclusion: Methanol is not considered carcinogenic based on the results of this mouse study.
Reliability: (2) valid with restrictions
14-MAR-2001
(58)

Species: mouse Sex: male/female
Strain: B6C3F1
Route of admin.: inhalation
Exposure period: 20 hours/day
Frequency of treatment: daily for one year
Post. obs. period: none
Doses: 0, 10, 100, 1000 ppm
Control Group: yes
NOAEL: >= 100 ppm
LOAEL: = 1000 ppm
Method: other

Year:	1986 GLP: no data
Test substance:	other TS
Method:	Thirty mice/sex per group were used in this study. Ten/sex per group sacrificed at 6 months and remainder at 12 months. The parameters evaluated were body weight, food consumption, urinalysis, clinical signs, hematology, histopathology, clinical chemistry and survival.
Remark:	This chronic inhalation study design was similar to standard chronic studies in mice except the length of the daily exposure was much longer than normal (20 vs 6 hrs). The design offered little time for normal clearance from the body as exposure was essentially continuous (not normal for industrial exposure or consumer use). The decrease food consumption and increase in body weight do not make sense.
Result:	In both male and females exposed at 1,000 ppm a statistical significant increase in body weight gain was noted with smaller changes at lower doses at 6 month but no statistical significant effect were noted at 12 months. One mouse died and one was sacrificed during the study (100 ppm). Food consumption was reduced in female mice, but it had no effect on body weight gain. A statistical significant increase in fatty degeneration of the liver in male mice was noted at 1,000 ppm, but fatty livers were noted in all other groups including the control males. No treatment-related effects were reported for, hematology, antibody test, urinalysis, serum or biochemical tests.
Conclusion:	The NOAEL in mice appears to be 100 ppm with a statistical significant increase in body weight gain, fatty livers (males) and decrease in food consumption (females) noted at 1,000 ppm.
Reliability:	(2) valid with restrictions
14-MAR-2001	
(58)	

Species:	monkey Sex: female
Strain:	Macaca Fascicularis
Route of admin.:	inhalation
Exposure period:	21 hours/day
Frequency of treatment:	daily up to 21 days
Post. obs. period:	none
Doses:	0, 3000, 5000, 7000, 10000 ppm
Control Group:	yes
NOAEL:	= 3000 ppm
LOAEL:	= 5000 ppm
Method:	other
Year:	1986 GLP: no data
Test substance:	Methanol, Junsei Chemical Co.
Method:	The monkeys were exposed for 21 hrs/ day. Exposure was for 6 days at 10,000 and 7,000 ppm, 14 day at 5,000 ppm , and 21 days at 3,000 ppm. Parameters evaluated included the eye, clinical signs, hematology, histopathology, clinical

chemistry and survival.

Remark: This was a pilot study to set test doses for larger sub-acute inhalation study. The study is useful demonstrating the lack of effect on the eye, CNS effects in a dose related manner and nerve damage at higher doses. The design offered little time for normal clearance from the body as exposure was essentially continuous (not normal for industrial exposure or consumer use).

Result: Monkey exposed at 3,000 ppm methanol showed no adverse effects, but at 5,000 ppm and higher reduced movement, weak knees, involuntary movement, vomiting and dyspnea was reported. No change in clinical chemistry, but slight changes in the central nervous system (hyperplasia of reactive astrocytes in the basal ganglion) were reported at 3,000 ppm. Animals exposed at 5,000 ppm and higher had decreased blood pH (acidosis). The blood methanol level was 526 mg/dl and formic acid 121 mg/dl at 5,000 ppm methanol vs 8 mg/dl methanol and 3 mg/dl at 3000 ppm or lower. Animals exposed at 5,000 ppm had increased neutral lipids (liver function). Degeneration of basal ganglion in the central nervous system and fatty degeneration of the liver were reported in a dose-related manner at 3,000 ppm and higher. Body weight was decreased at 10,000 ppm. One death was reported at day 14 (5,000 ppm). No effects were noted at any test level on the eye or optic nerve.

Conclusion: The minimal effect level in monkey exposed to methanol for 21 hours per day was 3,000 ppm.

Reliability: (2) valid with restrictions

14-MAR-2001

(58)

Species: monkey Sex: female

Strain: Macaca Fascicularis

Route of admin.: inhalation

Exposure period: 21 hours/day

Frequency of treatment: 12, 29, 210 days

Post. obs. period: 12 months

Doses: 1000, 2000, 3000, 5000 ppm

Control Group: no

NOAEL: < 1000 ppm

LOAEL: = 1000 ppm

Method: other

Year: 1986 GLP: no data

Test substance: Methanol, Junsei Chemical Co.

Method: The study used 3 monkeys /group, except 2/group at 5,000 ppm. The daily exposure was 21 hrs/ days. Exposure was for 12 days at 5,000 ppm, 20 days for 2,000 and 3,000 ppm, and 7 months for 1,000 ppm. Recovery periods were for 1, 4, 6, 12 months. Parameters evaluated included eyes, body weight, clinical signs, hematology, histopathology, clinical chemistry and survival.

Remark: This inhalation study had a complex design. No

control group was used. The design offered little time for normal clearance from the body as exposure was essentially continuous (not normal for industrial exposure or consumer use).

Result: Monkeys exposed at 1,000, 2,000 and 3,000 ppm methanol showed no clinical signs, but at 5,000 ppm reduced movement, weak knees, involuntary movement, vomiting and dyspnea was reported. No changes in body weight, temperature, or ECG were reported in any group. No clinical chemistry, but slight changes in the nervous system were reported at 1,000 ppm. Animals exposed at 2,000 ppm and higher had decreased blood pH (acidosis), and increased GTP. Changes in blood pH return to normal during the recovery period. Pathological changes in the central nervous system were reported in a dose-related manner at 1,000 ppm and higher. One death was reported at day 5 (5,000 ppm). Slight partial atrophy of the optic nerve was noted at 3,000 ppm and higher, but it was questionable if it was due to treatment or not.

Conclusion: The minimal effect level in monkey exposed to methanol for 21 hours per day for up to 7 months was 1,000 ppm.

Reliability: (2) valid with restrictions

14-MAR-2001

(58)

Species: monkey Sex: male/female

Strain: Macaca Fascicularis

Route of admin.: inhalation

Exposure period: 6 hours/day

Frequency of treatment: 5 days/week for 4 weeks

Post. obs. period: none

Doses: 0, 500, 2000, 5000 ppm

Control Group: yes, concurrent vehicle

NOAEL: > 5000 ppm

Method: other

Year: 1987 GLP: yes

Test substance: other TS

Method: The study used 3 monkeys /sex/ group. Parameters evaluated included, ophthalmoscopic exam, body weight, clinical signs, organ weights, histopathology and survival

Remark: A good screening study. Daily exposure was 6 hours / day the normal daily exposure length for inhalation studies evaluating potential workplace exposure.

Result: Actual exposure 0, 520, 1980 and 5010 ppm. All animals survived. No clinical signs were noted in monkeys. There was no treatment related effects on body weight. No ocular abnormalities were noted at the terminal ophthalmoscopic exam. No treatment related histopathological effects (35 tissues)were noted, but spleen weights were increased in the high dose female monkeys. No other organ weight effects were noted.

Test substance: Methanol from Celanese Corporation 99.85% pure

Conclusion: Monkeys were exposed to up to 5,000 ppm (6 hr/d, 5d/wk for 4 weeks) showed no treatment related effects, including the

	ophthalmoscopic exam. Overall the results support the use of the present TLV for methanol
Reliability:	(1) valid without restriction
14-MAR-2001	
(4)	

Species:	monkey Sex: female
Strain:	Macaca Fascicularis
Route of admin.:	inhalation
Exposure period:	22 hours/day
Frequency of treatment:	7 days/week for 2.5 years
Post. obs. period:	none
Doses:	0, 10, 100, 1000 ppm
Control Group:	yes
NOAEL:	<= 10 ppm
LOAEL:	= 10 ppm
Method:	other
Year:	1986 GLP: no data
Test substance:	Methanol, Junsei Chemical Co.
Method:	There were 8 female monkey/group, except for 2/group at 5,000 ppm. Sacrifices were conducted 7 month (2/group), 19 month (3/group), and at 30- month (3/group). Parameter evaluated included eye, body weight, clinical signs, hematology, histopathology, clinical chemistry and survival.
Remark:	The design offered little time for normal clearance from the body as exposure was essentially continuous (not normal for industrial exposure or consumer use).
Result:	Monkeys exposed at all level showed no clinical effects, but slight pathological changes in the nervous system (hyperplasia of reactive astroglias in the white matter) and liver (fatty degeneration) were noted at the seven month sacrifice). Slight blood changes were noted at 7 months in the blood in all but the 10-ppm group. In the 1000 ppm monkeys slightly abnormal ECG and blood pH were reported at 7 months. At the 19-month sacrifice the same nervous system and liver effects reported at 7 months were noted. At the final sacrifice hyperplasia of reactive astroglias (white matter) and liver (fatty degeneration) effects were noted all groups but the effect was less in the 10 and 100-ppm monkeys. The nervous system effects were thought to be transient and reversible after exposure was terminated, because no correlation with dose level, period of exposure and the magnitude of the effect. Slight liver and kidney effects were seen at 1000ppm and kidney effects only at 100 ppm
Conclusion:	The minimal effect level in monkey exposed to methanol for 22 hours per day for up to 7 months was 10 ppm. Repeated exposure may lead to transient pathological effects on the central nervous system.
Reliability:	(2) valid with restrictions
14-MAR-2001	
(58)	

Species:	rats	Sex: male/female
Strain:	Sprague-Dawley	
Route of admin.:	gavage	
Exposure period:	90 days	
Frequency of treatment:	daily	
Post. obs. period:		
Doses:	0, 100, 500, 2500 mg/kg bw	
Control Group:	yes	
NOAEL:	> 500 mg/kg bw	
LOAEL:	= 2500 mg/kg bw	
Method:	EPA	
Year:	1986	GLP: no data
Test substance:	Methanol	
Method:	The U.S. EPA Office of Solid Waste sponsored a 90-day subchronic testing of methanol in Sprague-Dawley rats (30/sex/dose). Six weeks after dosing, 10 rats/sex/dose group were sacrificed, the remaining rats were sacrificed at 90 days.	
Remark:	There were no differences between dosed animals and controls in body weight gain, food consumption, gross or microscopic evaluations. Elevated levels of SGPT, SAP, and increased, but not statistically significant, liver weights in both male and female rats suggest possible treatment-related effects in rats dosed with 2500 mg methanol/kg/day despite the absence of any histopathologic lesions in the liver. Brain weights in high-dose group males and females were significantly less than those of the control group.	
Conclusion:	Based on these findings, 500 mg/kg/day of methanol is considered a NOAEL in rats	
Reliability:	(2) valid with restrictions	

22-MAR-2001
(86)

5.4 Repeated Dose Toxicity (Added Remarks)

Remark:	In a limited study, Sprague Dawlev rats were exposed to airborne methanol concentrations of 200, 2,000 or 10,000 ppm for 6 hours per day, 5 days per week for as long as six weeks This caused no signs of lung inflammation or irritation. Histologic analyses of lung tissue were not conducted.

22-MAR-2001
(92)

Remark:	In an old Russian study, rabbits exposed to 61 mg/m3 (~50 ppm)methanol for six months (duration of exposure per day not given) were reported to have ultrastructural changes in the photoreceptor cells and Mueller fibers. Not enough information to be useful.

22-MAR-2001
(21)

Remark: Two dogs were exposed to about 13,000 mg/m3 (10,000 ppm) methanol for about three minutes at hourly intervals eight times daily for l00 days, a total of 800 brief exposures. Both dogs were reported to have survived the exposure and exhibited no symptoms or unusual behavior or visual toxicity attributable to methanol poisoning. Old, of little value.

22-MAR-2001
(71)

Remark: In an old study exposed four dogs were exposed to airborne concentrations of methanol from 585 to 650 mg/m3, eight hours per day, seven days per week for 379 days in a continuously ventilated chamber. Hematological determinations and ophthalmoscopic examinations were conducted. No adverse effects of any kind were reported. Old, of little value.

22-MAR-2001
(70)

Remark: In a old russian study rats received oral doses of 10, 100, or 500 mg/kg/ day for one month and were reported to show liver changes characterized by focal proteinic degeneration of hepatocytic cytoplasm. Old, of little value.

22-MAR-2001
(75)

Remark: Methanol was used as a solvent in an oral lifetime drinking water study of malonaldehyde in swiss mice. Three different levels of methanol were used in drinking water as controls (0.222, 0.444, 0.889%). The only effect noted was a reported increase in lymphomas in the two highest levels of methanol treated mice. The incidence rate of lymphomas was within historical control for the lab. An evaluation of a lifetime exposure to methanol by the oral route.

22-MAR-2001
(5)

Remark: Methanol was used as a solvent in a dermal study in hairless mice exposed topically to retinoic acid in methanol. The treatment was daily for 30 weeks. At the end of 55 weeks no skin tumors were noted in the methanol-only treated animals and no treatment related effects on the skin were noted. An evaluation of a chronic exposure to methanol by the dermal route.

22-MAR-2001
(1)

Remark: Methanol was used as a solvent in a life time skin painting study of malonaldehyde in swiss mice. The mice were treated 3 times a week with a dose of 0.05 ml of methanol. No treatment related effects on the skin, including skin tumors were noted. An evaluation of a lifetime exposure to methanol by the dermal route.

22-MAR-2001
(5)

5.5 Genetic Toxicity 'in Vitro'

Type:	Ames test
System of testing:	TA 98, TA100, TA1535, TA1537, TA1538, WP2uvrA
Concentration:	5, 10, 50, 100, 5000, 1000, 5000 ug/plate
Cytotoxic Conc.:	none
Metabolic activation:	with and without
Result:	negative
Method:	other
Year:	1985 GLP: no data
Test substance:	other TS
Remark:	See: DeFlora S.: (1982) Study of 106 organic and inorganic compounds in Salmonella/microsome test. Carcinogenesis, 2, 283-298.Methanol not active in TA98, TA100, TA1635, TA1537 or TA1538 with or without activation.
Test substance:	Methanol-Wacko Pure Chemical Industry
Conclusion:	Methanol is not mutagenic in this assay.

Reliability: (2) valid with restrictions Reliability
15-MAR-2001
(74)

Type: Cytogenetic assay
System of testing: Chinese hamster
Concentration: 0, 7,1, 14.3, 28.5 mg/ml
Cytotoxic Conc.: 28.5 mg/ml inhibit cell growth by 50%.
Metabolic activation: with and without
Result: negative
Method: other
Year: 1983 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: Methanol was dissolved in culture medium with or without 10% serum. Rats S9 was used for metabolic activation. Don cells from lung of Chinese hamster were used in this study. 200 chromosome were checked per dose level. Cells evaluated at 6, 24 and 48 hours after treatment.
Remark: Methanol produced an increase in SCE without metabolic activation at 28.5 mg/ml.
Result: Methanol did not induce chromosomal aberrations in this test.
Conclusion: Methanol is not mutagenic in this assay.
Reliability: (2) valid with restrictions
15-MAR-2001
(58)

Type: Escherichia coli reverse mutation assay
System of testing:
Concentration: 0. 10, 50, 100, 1000, 5000 ug/plate
Cytotoxic Conc.: none
Metabolic activation: with and without
Result: negative
Method: other
Year: 1983 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: Rat liver S9 was used for metabolic activation.
Result: Methanol did not cause an increase in mutations in E. coli.
Conclusion: methanol is not mutagenic in this assay.
Reliability: (2) valid with restrictions
15-MAR-2001
(58)

Type: Mammalian cell gene mutation assay
System of testing: Chinese hamster V79
Concentration: 0, 15.8, 31.7, 47.4, 63.3 mg/ml
Cytotoxic Conc.: 63.3 mg/ml (inhibit 70%).
Metabolic activation: with and without

Result: negative
Method: other
Year: 1983 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: Methanol was dissolved in culture medium. Rat liver S9 was used in this test. Cells were treated for 6 days and then evaluated.
Result: Methanol did not increase frequency of gene mutations in this assay.
Conclusion: methanol was not mutagenic in this test.
Reliability: (2) valid with restrictions
15-MAR-2001
(58)

Type: Salmonella typhimurium reverse mutation assay
System of testing:
Concentration: 0, 10, 40, 100, 1,000, 5000 ug/plate
Cytotoxic Conc.: none
Metabolic activation: with and without
Result: negative
Method: other
Year: 1983 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: Rat liver S9 was used in this assay. Plates incubated for 2 days at 37C.
Result: Methanol did not inhibit cell growth or increase the number of reverse mutation colonies.
Conclusion: Methanol was not mutagenic in this assay.
Reliability: (2) valid with restrictions
15-MAR-2001
(58)

Type: Yeast gene mutation assay
System of testing: Neurospora crassa
Concentration: unknown
Cytotoxic Conc.: unknown
Metabolic activation: without
Result: negative
Method: other
Year: 1984 GLP: no data
Test substance: no data
Reliability: (1) valid without restriction
15-MAR-2001
(3)

5.5 Genetic Toxicity 'in Vitro'(Added Remarks)

Remark: The in vitro induction of micronuclei in chinese hamster V79 cell was analyzed after exposure of the cells to 50 ul/ml of ethanol, methanol, butanol or propanol. None of the 4 alcohols induced micronuclei

22-MAR-2001
(48)

Remark: Methanol did induce chromosomal changes in Aspergillus. It did not induce sister chromatic exchanges in Chinese hamster cells in vitro, but caused significant increases in mutation frequencies in L5178Y mouse lymphoma cells.

22-MAR-2001
(95)

Remark: A report of increased mutation frequency in L5178Y mouse lymphoma cells with activation was reported in an abstract

22-MAR-2001
(53)

Remark: Methanol has been reported to show negative results in all in vitro sister chromatid exchange tests, cell transformation assays and Neurospora crassa tests, when assayed without exogenous metabolic activation

22-MAR-2001
(89)

5.6 Genetic Toxicity 'in Vivo'

Type: Cytogenetic assay
Species: mouse Sex: male
Strain: other
Route of admin.: inhalation
Exposure period: 6 hours. 5 days
Doses: 0, 800, 4000 ppm
Result: negative
Method: other (calculated)
Year: 1990 GLP: no data
Test substance: other TS
Remark: EPA study conducted in a government laboratory.
Result: No genotoxic effects noted (NOAEL(NOEL) (8,000 ppm). No evidence of treatment induced SCE, chromosome aberration or micronucleus in lung cell. No evidence of treatment induced synaptoneal complex damage in spermatocytes. No evidence of treatment induced increased frequencies of micronucleus in blood cells
Test substance: Methanol, 99.9% pure Fisher Scientific
Conclusion: No cytogenetic effects (SCE, CA, Mn) were seen in male mice exposed for 5 days to 400 or 8, 000 ppm methanol (blood cells, lung cell, testicular germ cell).
Reliability: (1) valid without restriction
14-MAR-2001
(19)

Type:	Micronucleus assay		
Species:	mouse	Sex: male	
Strain:	ICR		
Route of admin.:	gavage		
Exposure period:	24 hours		
Doses:	1.05, 2.11, 4.21, 8.41 g/kg bw		
Result:	negative		
Method:	other		
Year:	1983	GLP: no	
Test substance:	Methanol, Junsei Chemical Co.		
Method:	Six animals per group were used. Volume of dose was 20 ml/kg. The mice were killed 24 hours after dosing. Bone marrow was taken from the femur and 1000 multi-stationable red blood cells were checked.		
Result:	Under conditions of the test, methanol didn’t cause an increase in micronucleus at any test level , including the LD50		
Reliability:	(2) valid with restrictions		

15-MAR-2001
(58)

5.6 Genetic Toxicity 'in Vivo'(Added Remarks)

Remark:	In a gavage study normal and folate deficient mice were given 0, 300, 600, 1200 or 2500 mg/kg of methanol in 4 daily doses. No indication of genotoxic response to methanol was reported in the normal or folate deficient mice

22-MAR-2001
(59)

5.8 Toxicity to Reproduction

Type:	Fertility		
Species:	monkey	Sex: female	
Strain:	no data		
Route of admin.:	inhalation		
Exposure Period:	2.5 hrs		
Frequency of treatment:	daily - 365 d		
Premating Exposure Period			
male:	none		
female:	120 d		
Duration of test:	365 d		
Doses:	0, 200, 600, 1800 ppm		
Control Group:	yes		
NOAEL Parental:	> 1800 ppm		
Method:	other		
Year:	1999	GLP: yes	

Test substance: Methanol 99.05 pure, HPLC Fisher Scientific
Method: The study used 11 or 12 female monkeys/per group. Exposure was 2.5 hr/day, 7 days/week before and during pregnancy. Parameters evaluated included body weight, clinical signs, menstrual cycle, timed matings, pregnancy observations and survival. Blood methanol, plasma formate, and serum folate was determined every other week. Delivery exams were conducted on offspring. In addition body weight, clinical signs, and survival were also evaluated.
Remark:. An excellent well conducted study in a species that is more closely associated with humans as far as methanol toxicity is concerned. Study was sponsored by EPA, auto companies and API. Study was reviewed by an outside expert panel, who agreed with the study author's conclusions.
Result:
The weights of all females were quite stable during the study. Mean weight gain during pregnancy varied from 1.3 kg to 1.8 kg across all exposure groups. Clinical observations did not indicate the presence of overt toxicity in the adult females, and none exhibited a pattern of responses inductive of fine-motor incoordination due to methanol exposure. Methanol exposures did not affect the size of the offspring at birth, the average birth weight, crown-rump length, and head size of infants in the methanol-exposure groups.
Conclusion:
Chronic methanol exposures for up to 1 year did not cause overt maternal toxicity in m. fascicularis females. The the ability of females give birth to healthy live-born infants were also unaffected. Methanol exposures were associated with a reduction in the length of pregnancy (not dose dependent). No decrease in offspring birth size was observed.
Reliability: (1) valid without restriction
14-MAR-2001
(17)

Type: Two generation study
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure Period: 20 hr
Frequency of treatment: daily
Premating Exposure Period
male: P= 8-16 wks, F1=0-14 wks, F2=0-8 wks
female: P=8-16 wks, F1=0-21 wks, F2=0-8 wks
Duration of test: 365 days
Doses: 0, 10, 100, 1000 ppm
Control Group: yes
NOAEL Parental: = 100 ppm
NOAEL F1 Offspr.: = 100 ppm
NOAEL F2 Offspr.: = 100 ppm
LOEL parental : = 1000 ppm
LOEL F1 offspring : = 1000 ppm
Method: other

Year:	1985 GLP: no data
Test substance:	Methanol, Junsei Chemical Co.
Method:	Thirty /sex per group were used in the F0 generation. Observations were made in the F0, F1, and F2 generation. Parameters evaluated included body weight, food consumption, clinical signs, organ weights, histopathology and survival. In the offspring general observation at birth, organ check, organ texture, genital function and movement were evaluated.
Remark:	This 2-generation study design was similar to standard 2-generation studies in rats except the length of the daily exposure was much longer than normal (20 vs 6 hrs). The design offered little time for normal clearance from the body as exposure was essentially continuous (not normal for industrial exposure or consumer use).
Result:	No treatment related effects were reported for clinical signs, but body weight was decreased after 7 weeks in rats exposed to 1,000 ppm (significant in male, not significant in females). Food consumption was also decreased in the top dose animals. No treatment related effects on sexual cycle, pregnancy, delivery or reproductive capacity was observed. Offspring appeared normal and no histopathological effects were noted. The F1 rats showed no treatment-related effects on body weight, food/ water consumption or general movement. The high dose male offspring had a significant increase in the rate of early descensus testis. Brain weights were statistically significantly reduced in the high dose males and females at 8 weeks of age as well asin males sacrificed at 16 weeks and possible females at 24 weeks. The F 2 rats showed no treatment-related effects on body weight, food/ water consumption or general movement. The high dose male offspring had a significant increase in the rate of early descensus testis. Brain, hypophysis and thymus weights were statistically significantly reduced in the 1,000 ppm males and females at 8 weeks of age.
Conclusion:	Inhalation exposure had some slight treatment-related effects in rats exposed at 1,000 ppm in a 2-generation study. The effects did not effect reproductive performance. 100 ppm was a NOEL
Reliability:	(2) valid with restrictions
14-MAR-2001	
(58)	

5.8 Toxicity to Reproduction (Added Remarks)

Remark:	Mature male rats (Sprague-Dawley) were exposed to methanol concentrations of 200, 2,000, or 10,000 ppm for one, two, four, six weeks and examined them for alteration in circulating free testosterone, lutenizing hormone and follicle stimulating hormone. Significantly decreased levels of circulating free testosterone were observed among rats exposed at 200 ppm for 2 and 6 weeks and 6 weeks at 2,000 ppm. The high dose group (10,000-ppm) showed no change. The authors interpreted this as evidence that methanol exposure had lowered testicular production of testosterone. In addition, significant increases in circulating LH were

observed after six weeks of exposure to 10,000 ppm. No changes in follicle stimulating hormone levels were observed.

Reliability: (2) valid with restrictions
22-MAR-2001
(18)

Remark: The potential toxic effects of methanol vapors on testicular production of testosterone and the morphology of testes were investigated using normal or methanol-sensitive folate-reduced rats. Methanol inhalation at the level of 200 ppm, for up to six weeks (8 hours/day, 5 days/week), did not reduce serum testosterone levels in normal rats. Testes isolated from methanol-exposed (200 ppm) rats had the same capability as those from air-exposed rats in synthesizing testosterone whether testes were incubated in the absence or presence of hCG. The testes-to-body weight ratio of rats exposed up to 800 ppm methanol for up to 13 weeks (20 hours/day, 7 days/week) were not different from those of the air-exposed rats.

Reliability: (2) valid with restrictions
22-MAR-2001
(49)

Remark: Two experiments were conducted to evaluate the acute effects of inhaled methanol on serum hormones associated with reproductive function in the male rat. In the first experiment, rats exposed to methanol (0, 200, 5000 and 10,000 ppm) for 6 hours were killed at the end of the exposure period or the following morning. The effect of the handling associated with placing the rat in the exposure chamber was also evaluated by comparing hormonal changes in sham- and methanol-exposed groups acclimated for two weeks with groups that were not acclimated. Prior handling resulted in an increase in serum lutenizing hormone greater in the non-acclimated groups than in the acclimated group. In the second experiment, groups of acclimated and non-acclimated rats were exposed to 0 or 5000 ppm methanol for 1, 2 and 6 hours and killed immediately after removal from the chamber. Serum lutenizing hormone, testosterone and follicle stimulating hormone values were not different in sham- vs methanol-exposed rats at any time point. As in experiment 1, an effect of prior handling was noted. In general, the concentrations of these hormones and serum prolactin in the non-acclimated rats were greater than those observed for acclimated rats. Methanol exposure resulted in increased prolactin concentrations under both handling conditions

Reliability: (2) valid with restrictions
22-MAR-2001
(24)

5.9 Developmental Toxicity/Teratogenicity

Species: rat Sex: female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 7 hours/day
Frequency of treatment: daily
Duration of test: day 1-19 of gestation
Doses: 0, 5000, 10000, 20000 ppm
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxicity: >= 10000 ppm
NOAEL Teratogenicity: = 10000 ppm
LOAEL Maternal Toxicity: <= 20000 ppm
LOAEL Teratogenicity : <= 20000 ppm
Method: other
Year: 1985 GLP: no
Test substance: Reagent Grade Methanol -Matheson, Colemen & Bell Manuf. Chemists
Method: Pregnant females, 15 per group, except 13/ group at 5,000 ppm were exposed for 7 hrs/ day, on days 1-19 of gestation. Parameters evaluated included body weight, food consumption, clinical signs, and survival. Blood methanol level, corpora lutea, dead, resorbed, malformed fetuses [half visceral, half skeletal] observations were also made.
Remark: Different incidences of visceral malformations were reported in the text than were reported in the tables. The occurrence of maternal toxicity in the significantly affected group compromises an interpretation of the teratogenic effects as being solely the result of in utero methanol exposure.
Result: The highest concentration of methanol produced slight maternal toxicity (unsteady gait) and a high incidence of congenital malformations, predominantly extra or rudimentary cervical ribs and urinary or cardiovascular defects. Blood methanol increased with dose (1.00, 2.24 and 8.65 mg/ml}. The fetal observations that were significantly different from control ($p<0.05$) at 20,000 ppm are: Number of litters(fetus) with visceral and skeletal malformation, the percentage of litters with abnormal fetuses and the percentage of normal fetus. A non statistical increase in malformation was also reported at 10,000 ppm. No significantly differences from control ($p<0.05$) was noted at any of the lower doses.
Conclusion: The highest level of methanol produced slight maternal toxicity and a significant increase in congenital malformations. A non statistical increase in malformation was also reported at 10,000 ppm
Reliability: (2) valid with restrictions
15-MAR-2001
(55)

Species: rat Sex: female
Strain: Sprague-Dawley
Route of admin.: inhalation
Exposure period: 20 hours/day
Frequency of treatment: daily
Duration of test: day 7-17 of gestation

Doses: 0, 200, 1000, 5000 ppm
Control Group: yes
NOAEL Maternal Toxicity: > 1000 ppm
NOAEL Teratogenicity: > 1000 ppm
LOAEL Maternal Toxicity : <= 5000 ppm
LOAEL Teratogenicity : <= 5000 ppm
NOAEL Embryotoxicity : >= 1000 ppm
Method: other
Year: 1986 GLP: no data
Test substance: Methanol, Junsei Chemical Co.
Method: Thirty-six pregnant rats per group were used in this study. Twenty were sacrificed/group at day 20 and 10/group were allowed normal delivery. Parameters evaluated included body weight, food consumption, clinical signs, organ weight, histopathology and survival. The number of fertilized corpus luteum, implantation, living and death fetuses, were also determined. Visceral or skeleton evaluations were conducted on each litter. Offspring from the normal delivery were subjected to general observation at birth, organ check, organ texture, genital function and movement. The F1 were bred and all sacrificed at day 20, litter evaluated as above.
Remark: Similar to standard teratogenicity study in rats except the length of the daily exposure was much longer than normal (20 vs 6 hrs little time for clearance as exposure was essentially continuous) and some pregnant rats were allowed to deliver normally.
Result: Dams exposed at 5,000 ppm had decreased rate of body weight gain, decreased food/water consumption, and one death (plus one sacrificed). Increased embryo mortality, decreased fetal weight, increase in septal defects, obstructed vertebro-costal foramen, cervical ribs, excess sublingual foramen nervosa, delayed fetal growth and reduced ossification were noted in the high dose fetuses. In the normal delivery groups decreased food/water consumption, and delayed pregnancy (0.7 days) were reported in the high dose. Pups from the high dose group had increased early mortality, lower birth weights, lower postnatal body weight and a slight decreased water consumption (no effect on food consumption). The high dose male offspring sacrificed at 8 weeks had decreased brain, thyroid, thymus, testes weight and increased in hypohysis weight. The high dose females had reduced brain and thymus weights. No histopathological effects were noted in these organs, except hemilateral thyroid defects
Conclusion: Inhalation exposure demonstrated treatment-related effects in rats and their fetuses exposed at 5,000 ppm. Fetal toxicity, visceral/skeleton effects were seen in the fetuses exposed at 5,000 ppm , but this dose was maternally toxic. Methanol is not considered teratogenic in this study. 1,000 ppm was a NOAEL for both the dam and the fetus.
Reliability: (2) valid with restrictions
15-MAR-2001
(58)

Species: mouse Sex: female

Strain: CD-1
Route of admin.: inhalation
Exposure period: 7 hours/day
Frequency of treatment: day 6-15 of gestation
Duration of test: 20 days
Doses: 0, 1000, 2000, 5000, 7500, 10000, 15000 ppm
Control Group: yes
NOAEL Maternal Toxicity: > 15000 ppm
NOAEL Teratogenicity: = 1000 ppm
NOAEL Embryotoxicity : = 1000 ppm
LOAEL Teratogenicity : = 2000 ppm
LOAEL Embryotoxicity : = 2000 ppm
Method: other
Year: 1993 GLP: no data
Test substance: Methanol , High purity Optima grade Fisher Scientific
Method: Blood methanol concentrations were determined on gestation days 6, 10, and 15. Fetus were examined for number of implantation sites, live/dead fetuses, and resorptions. Fetuses were examined externally and weighed as a litter. Half of each litter was examined for skeletal morphology/ Internal soft tissue anomalies.
Remark: Study design is complex. Only 3 chambers were used and exposures were staggered. Number of animal used appeared to be adequate, but it was hard to tell exact number used, as data was listed as litters per treatment group. Some animals were removed for plasma methanol determinations.
Result: One dam died in each of the 7,500, 10,000, and 15,000 ppm methanol exposure groups. The methanol exposed dams gained less weight than did unexposed dams fed ad libitum. Significant increases in the incidence of exencephaly and cleft palate were observed at 5,000 ppm and above, increased embryo/fetal death at 7,500 ppm and above (including an increasing incidence of full- litter resorptions), and reduced fetal weight at 10,000 ppm and above. A dose-related increase in cervical ribs or ossification sites lateral to the seventh cervical vertebra was significant at 2,000 ppm and above. Methanol plasma levels increased with dose. No signs of maternal toxicity were noted.
Conclusion: The NOAEL for the developmental toxicity in this study was 1,000 ppm.
Reliability: (2) valid with restrictions
15-MAR-2001
(66)

Species: mouse Sex: female
Strain: CD-1
Route of admin.: inhalation
Exposure period: 6 hours/day
Frequency of treatment: 7-9 and day 9-11, day 6-15
Duration of test: 20 days
Doses: 0, 5000, 10000, 15000 ppm
Control Group: yes

NOAEL Maternal Toxicity: >= 10000 ppm
NOAEL Teratogenicity: = 5000 ppm
LOAEL Maternal Toxicity : <= 15000 ppm
LOAEL Teratogenicity : = 10000 ppm
NOAEL Embryotoxicity : = 5000 ppm
Method: other
Year: 1993 GLP: no data
Test substance: Methanol -HPLC grade J.T. Baker
Method: The study used 17-27 pregnant mice per group. Parameters evaluated include body weight, clinical signs, and survival. The number of live/ dead fetuses, resorbed, and malformed fetuses[visceral] plus implant sites was also determined.
Remark: A pilot study was also reported in this paper that was used to set conditions for the main study. A good special study that examined certain time periods during gestation and the effect of methanol on neural tube defects.
Result: Neurological effects (ataxia, depressed motor activity circling, tilted heads) were noted in the 15,000 ppm dams only on days 1,2 and 3 of exposure (20, 10, 5%). Maternal body weights were decreased at day 17 at 15,000 ppm (high resorptions). Embryotoxicity was noted in fetuses (increased resorptions, reduced fetal weights, and/or fetal malformations) at 10,000 and 15,000 ppm level, while no observable adverse effects were seen in the 5,000 ppm group. Neural and ocular defects, cleft palate, hydronephrosis, deformed tails, and limb (paw and digit) anomalies were reported. Neural tube defects and ocular lesions occurred after methanol inhalation between GD 7-9, while limb anomalies were induced only during GD 9-11, cleft palate and hydronephrosis were observed after exposure during either period.
Conclusion: The highest level of methanol (15,000 ppm) produced slight maternal toxicity and embryotoxicity. Teratogenicity and embryotoxicity was also reported at 10,000 ppm, but not 5,000 ppm.
Reliability: (2) valid with restrictions
15-MAR-2001
(13)

Species: monkey Sex: female
Strain: Macaca Fascicularis
Route of admin.: inhalation
Exposure period: 2.5 hours/day
Frequency of treatment: daily
Duration of test: 120 days
Doses: 0, 200, 600, 1800 ppm
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxicity: > 1800 ppm
NOAEL Teratogenicity: > 1800 ppm
Method: other
Year: 1999 GLP: no data
Test substance: Methanol , High purity HPLC grade Fisher Scientific

Method: Eleven or 12 female monkey per group were used in this study. Pregnancy observations and delivery exam were conducted. In addition body weight, clinical signs, and survival were also

evaluated.

Remark: An excellent well conducted study in a species that is more closely associated with humans as far as methanol toxicity is concerned. Study was sponsored by EPA, auto companies and API. Study was reviewed by an outside expert panel, who agreed with the study author's conclusions.

Result: The weights of all females were quite stable during the study. Mean weight gain during pregnancy varied from 1.3 kg to 1.8 kg across all exposure groups. Clinical observations did not indicate the presence of overt toxicity in the adult females, and none exhibited a pattern of responses indicative of fine-motor incoordination due to methanol exposure. Methanol exposures were associated with a reduction in the length of pregnancy, thus shortening the gestation period of the offspring, but did not affect the size of the offspring at birth, the average birth weight, crown-rump length, and head size of infants in the methanol-exposure groups. Neurobehavioral testing suggested possible effects in two tests, but the effects was not concentration dependant or a significant overall effect across the methanol groups. Another observation, not judged a treatment related effect, was a serve wasting syndrome in two female offspring in the high dose methanol group. These two observations were not considered to be treatment related.

Conclusion: Chronic methanol exposures for up to 1 year did not cause overt maternal toxicity in m. fascicularis females. Methanol exposures were associated with a reduction in the length of pregnancy (not dose dependent). No decrease in offspring birth size was observed. No obvious birth defects were noted.

Reliability: (1) valid without restriction
15-MAR-2001
(16)

5.9 Developmental Toxicity/Teratogenicity (Added Remarks)

Remark: Rats were dosed by gavage during Days 1-8 of pregnancy at 0, 1.6, 2.4, or 3.2 g methanol/kg/day. Animals were killed on Days 9, 11, or 20 of pregnancy, and maternal, embryonic, or fetal parameters were assessed. No treatment related effect on estradiol, progesterone, lutenizing hormone and prolactin was observed. Reductions in pregnant uterine and implantation site weights were seen on Day 9, but no effects on embryo/fetal survival or development were noted. The 3.2 g/kg/day dose of methanol produced a reduction in body weight gain by Day 9, which may be considered an indication of non-specific maternal toxicity. No effect on Day 11 or Day 20 on embryo-fetal survival, or development was observed.

22-MAR-2001
(26)

Remark: A gavage study compared well-nourished and malnourished in rats given 2.5 g/kg methanol from day 6-15 of gestation. An increase in skeletal malformation, primarily cervical extra ribs was noted in the methanol treated rats when compared to controls. Malnutrition did not increase the incidence of malformations, but fetal growth was retarded. This is only a single dose study and the 2.5 g/kg/day dose is above the lethal dose in humans.

22-MAR-2001
(27)

Remark: Pregnant rats received a drinking solution containing 2% methanol during gestational days 15 through 17 or during days 17 through 19. The average methanol consumption was 2.5 g/kg/day. Behavioral effects such as increased latency to suckling behavior, and a lower efficiency in seeking and reaching their home area were reported. Methanol treatments did not affect the dam (duration of gestation, weight gain or maternal behavior on the day of parturition) or fetus (litter size, birthweight, weight gain, infant mortality or day of eye opening). The behavioral effects noted in this study occur at tissue levels of methanol lower than those associated with teratogenesis in the study by Nelson et al (1985), and may be of potential significance. However, a maternal exposure in this study was 2.5 g/kg/day, which is above the lethal dose in humans.

23-MAR-2001
(42)

Remark: No significant changes in neurobehavioral and neurophysiological development in the offspring of rats exposed to 15,000 ppm methanol vapors (7 hours daily between gestational days 7 and 19) were noted in this study.

23-MAR-2001
(79)

Remark: In an inhalation study in rats exposed to 4,500 ppm for 6 hours per day for day 6 of gestation through day 21 postnatal subtle behavioral changes were reported in both dams and neonates.

23-MAR-2001
(80)

Remark: In a study that evaluated the critical periods for the developmental toxicity of methanol, pregnant CD-1 mice were exposed to 10,000 ppm methanol or filtered air for 7 hr/day on 2 consecutive days during gestation day 6-13, or to single day (7 hr) exposures to 10,000 ppm methanol during gestation day 5-9. Peak maternal blood methanol concentration (at the end of exposure) was ~4 mg/ml, and methanol was cleared from maternal blood within 24 hr. Some fully resorbed litters

were observed with 2-day methanol exposures on gestation day 6-7 or 7-8, or 1-day exposure on gestation day 7. With 1-day methanol exposure on gestation day 7, the number of live fetuses was lower than with exposure on any other day. Cleft palate, exencephaly, and skeletal defects were the fetal anomalies observed. These results indicate that gestation and early organogenesis represent a period of increased embryonal sensitivity to methanol in mice.

23-MAR-2001
(65)

Remark: Cephalic neural tube defects were observed in near-term mouse fetuses following maternal inhalation of methanol at a 15,000 ppm for 6 hr/day on gestation day 17. Neural tube defects, chiefly exencephaly, occurred in 15% of fetuses (reduction or absence of multiple bones in the craniofacial skeleton, prematurely open eyelids, cataracts, and retinal folds). Following daily 6-hour maternal inhalation of 15,000 ppm methanol during gestation day 7-8, the cephalic neural fold margins were swollen, blunted, and poorly elevated on gestation day 8.5 and 9 relative to controls. Histopathology of exposed gestation day 8.5 embryos revealed microcephaly, reduced mitotic index in the embryonic neuroepithelium and groups of neural crest cells were displaced to the neural folds dorsal to the foregut. When examined on gestation day 9.5 and 10.5, maternal methanol exposure (15,000 ppm for 6 hr/day) during gestation day 7-9 resulted in stunting, delayed rotation, and microcepholy in over 90% of the affected embryos. Many 10.5-day-old embryos were edematous. Occult neural tube defects was present in at least 21 % of methanol-exposed embryos on gestation day 9.5 and 10.5. There were no apparent neural tube defects in control embryos at any stage of development. These data suggest 1) that exposure to high concentrations of methanol injures multiple stem cell populations in the neurulating mouse embryo and 2) that significant neural pathology may remain in older conceptuses even in the absence of gross lesions.

23-MAR-2001
(14)

Remark: Female mice were given 2.5-g/kg methanol by gavage for twice daily, gestation day 6-10 plus 400 or 1200 nmol folic acid for 5 weeks prior to mating through gestation. The marginal folic acid dietary treatment (400 nmol) resulted in low maternal liver, red cell and low fetal tissue folic acid concentrations. Marginal folic acid-methanol treatment resulted in an increase in the litters affected by cleft palate and exencephaly. These results show that marginal folate deficiency in pregnant dams significantly increases the teratogenicity of methanol.

23-MAR-2001
(35)

Remark: Female mice were fed one of three diets containing 400 (low), 600 (marginal, or 1,200 (adequate) nmol folic acid diet for 5 weeks prior to and following mating. On gestation days 6-15,

dams received distilled water or methanol at 2.0 or 2.5 g/kg body weight, twice daily. Maternal liver folate concentrations were lower in the low dietary folic acid groups than in the marginal and adequate groups; methanol did not affect maternal liver folate concentration at term. Maternal net gestational weight gain was lowest at the lowest dietary folate level but was not affected by methanol. Gravid uterus weights were lowest in the low dietary folic acid groups exposed to the high methanol dose and the number of live fetuses per litter was lowest in the low folic acid groups. Fetal body weights were lowest in the low folic acid groups and significantly lower in the methanol groups relative to vehicle treated animals. Fetal crown-rump lengths were shorter in the methanol-treated groups; this parameter was not affected by folic acid treatment. Both methanol and low dietary folic acid increased the incidence of cleft palate, with the highest number of affected litters in the low dietary folic acid group. These results support the concept that maternal folate status can modulate the developmental toxicity of methanol.

23-MAR-2001
(69)

Remark: Pregnant mice were gavaged with 0, 4.0, or 5.0 g/kg methanol split in two doses on gestation day 7, the most sensitive day for induction of skeletal alterations by methanol. These results demonstrate that maternal methanol exposure can alter segment patterning in the developing mouse embryo, producing posteriorization of cervical vertebrae

23-MAR-2001
(22)

Remark: In a pilot study methanol given (0.5, 1.0 or 2.0 %) in the drinking water on gestation day 6-15 induced developmental effects in folate deficent Long-Evans rats. Skeletal effects were seen at the top two doses

23-MAR-2001
(68)

DISCUSSION OF SPECIES DIFFERENCES

The toxicity of methanol varies greatly between different species, depending on the ability to metabolize formate. In such cases of slow metabolism of formate, fatal methanol poisoning occurs as a result of metabolic acidosis and neuronal toxicity, whereas, in animals that readily metabolize formate, CNS depression (coma, respiratory failure, etc.) is usually seen. Sensitive primate species (humans and monkeys) develop increased blood formate concentrations following high level methanol exposure, while resistant rodents, rabbits and dogs do not.

Humans (and non-human primates) are uniquely sensitive to methanol poisoning and the toxic effects in these species is characterized by formic metabolic acidosis, ocular toxicity, nervous system depression, blindness, coma and death. Nearly all of the available information on methanol toxicity in humans relates to the consequences of acute rather than chronic exposures.

A vast majority of poisonings involving methanol has occurred from drinking adulterated beverages and from methanol-containing products. Although ingestion dominates as the most frequent route of poisoning, inhalation of high concentrations of methanol vapor and percutaneous absorption of methanol could produce acute toxic effects.

An experimental study of dermal exposure with neat methanol in human volunteers for the purposes of estimating percutaneous absorption rates was conducted. A large percentage (mean of 68%) of the dermally-applied methanol evaporated within 60 minutes (Raabe et al., 1992). The maximum concentration of methanol in blood following an exposure to one hand lasting -20 min is comparable to that reached following inhalation exposures at a methanol concentration of 200 ppm, the threshold limit value-time weight average (TLV-TWA) (Batterman and Franzblau 1997).

The lethal dose of methanol for humans is not known for certain. The minimal lethal dose of methanol in the absence of medical treatment is estimated to be around 1 g/kg. The minimum dose causing permanent visual defects is also unknown. Two important constituents of human response to methanol appear to be (1) concurrent ingestion of ethanol, which slows the entrance of methanol into the metabolic pathway, and (2) hepatic folate status, which governs the rate of formate detoxification.

The symptom and signs of methanol poisoning in humans, usually will not appear until about 12 to 24 hours after exposure, and include visual disturbances, nausea, abdominal and muscle pain, dizziness, weakness and disturbances of consciousness ranging from coma to clonic seizures. Visual disturbances generally develop between 12 and 48 h after methanol ingestion and range from mild photophobia and misty or blurred vision to markedly reduced visual acuity and complete blindness. In extreme cases death can result. The principal clinical feature is severe metabolic acidosis largely attributed to the formic acid produced when methanol is metabolized.

The normal blood concentration of methanol in humans from endogenous sources is less than 0.5 mg/liter (0.02 mmol/liter), but dietary sources may increase blood methanol level. Generally, transient Central Nervous System (CNS) effects appear above blood methanol levels of 200 mg/liter (6 mmol/liter); ocular symptoms appear above 500 mg/liter (16 mmol/liter) and fatalities have occurred in untreated patients with initial methanol levels in the range of 1500-2000 mg/liter (47-62 mmol/liter). Metabolic acidosis and ocular toxicity characterize methanol toxicity in humans and monkeys, and appears to be due to the accumulation of formate. This accumulation is due to a deficiency in formate metabolism, which results from low hepatic tetrahydrofolate (H4 folate) levels. Humans and monkeys possess low hepatic H4 folate levels. This results in low rates of formate oxidation and accumulation of formate after large doses of methanol (Tephly-1991).

Acute inhalation of methanol vapor concentrations below 260 mg/m^3 or ingestion of up to 600 mg methanol by healthy or moderately folate-deficient humans does not result in formate accumulation above endogenous levels (Lee et al 1992, Leon et al., 1989).

Ocular toxicity, a well-recognized outcome of methanol poisoning in humans, correlates with formate accumulation in blood. Total folate levels were determined in human and rat retinal tissues and were found to be much lower than the levels in liver. However, folate levels in human retina were only 14%

of those determined in rat retina. The amount of 10- formyl tetrahydrofolate dehydrogenase (10-FDH) in human retina was approximately three times the amount found in rat retina. 10-FDH was found to be preferentially localized in the Mueller cells, which appear to represent the target for formate induced ocular toxicity. Formate oxidation reactions might serve two roles, first a protective role and then a role in methanol-induced toxicity in Mueller cells (Martinasevic etal., 1996).

Role of metabolism on the species differences

Animal tests were done over the years to obtain predictive information. The investigation of methanol toxicity in animals is somewhat difficult to correlate to human effect because normal rodents exposed to methanol do not display the metabolic acidosis and toxicity to the visual system that occurs in humans (Roe 1982, Tephly and McMartin 1984; World Health Organization 1997).

The monkey is most like man when it comes to formate level following exposure to methanol. Chronic methanol exposures (2.5 hours per day, 0, 200 600 or 1,800 ppm) for up to 1 year in *m. fascicularis* females was studied by Burbacher (1999). The study included measuring blood methanol and plamsa formate levels. While blood methanol levels increase with dose, no changes in blood formate levels were noted (Burbacher et al 1999). In a single inhalation exposure of a Rhesus monkey for 6 hours at 2000 ppm resulted in no increase in formate levels (Horton et al., 1992).

NEDO (1987) also exposed rats and primates to methanol by gavage and measured blood formate levels. Only at 3000 mg/kg were formate levels evaluated in both speces. Lower doses (25, 125 and 600 mg/kg) showed no elevation in blood formate. This is consistent with a toxic dose (optic injury, minimum lethal dose) in humans of about 1000 mg/kg (Roe 1982).

In a study by Andrews et al (1987) monkeys exposed to 5,000 ppm methanol for 6 hours per day, 5 days/week showed no treatment-related changes when compared to the controls.

NEDO (1987) exposed primates nearly continuously for up to 14 days (20+ hours a day, 7 day per week). In this nearly continuous sub-acute exposure study toxicity was noted at 5,000 ppm but not at 3,000 ppm. The toxicity observed at 5,000 ppm correlates with the increase in formate level observed at 5,000 ppm (but not at 3,000 ppm).

The difference in the response in the two studies is most likely due to the difference in exposure patterns. The NEDO studies used 20+ hours exposure per day. The half-life on methanol in the body is 1-3 hours (Burbacher et al., 1999). The NEDO study design gives a possibility for the build-up of methanol and formate in the body because of lack of clearance time. In the Andrews et al (1987) study exposure was for 6 hours per day with 18 hours for clearance. The difference in the response at 5,000 ppm in the two studies might be explained by the total daily dose between the two studies.

Using Haber’s law (CT=K) the total dose is much greater in the NEDO studies [NEDDO- 3,000 ppm (3,000 x 20 hrs =60,000 ppmhr), 5,000 ppm (5,000 x 20 hrs = 100,000 ppmhr)] verus [Andrews –5,000 ppm (5,000 x 6 hrs = 30,000 ppmhr)]. The results of this comparison suggest that between 60,000 ppmhr and 100,000 ppmhr there is formate build-up in the 14 day NEDO study, but the lack of clearance makes it difficult in the NEOD studies to get an accurate daily dose over time.

Burbacher et al (1999) evaluated the effects of methanol inhalation exposure on pregnant monkeys exposed at a top daily dose of 4500 ppmhr (1800 ppm x 2.5 hrs). The top dose in this study was a NOAEL. The Andrews et al (1987) data suggest the NOAEL could be higher than the top dose used by Burbacher et al (1999).

Incorporation of kinetic parameters and the fraction of inhaled methanol absorbed in humans and rodents into kinetic models indicate that an 8-hour exposure to 5,000 ppm methanol produced some very different results in different species.

The blood methanol level in the mouse is 13-18 times higher and in the rat it is 5 times higher than in humans exposed to the same 5,000 ppm inhaled level (Perkin et al., 1995). This species difference may also be related to the difference in response of pregnant animals to methanol. The mouse is the most sensitive showing developmental effects below a maternal toxic doses while the rat only responds at higher doses that are maternally toxic.

There is abundant data on the potential health effects of methanol in humans. Most information on the human health effects of methanol is derived from clinical observations following accidental or intentional ingestion of methanol. Methanol can be highly toxic, resulting in nausea, dizziness, metabolic acidosis, and toxicity to the visual system (including blindness), motor disturbances and even death in humans. Exposure of humans to 200 ppm does not increase blood formate levels or result in any toxicity (Lee et al., 1992).

The absorption of methanol is rapid following oral ingestion, inhalation of methanol vapor, or skin contact. High doses of methanol overwhelm the humans body's ability to remove a toxic metabolite (formate). When formate accumulates, toxicity occurs.

(1) Forbes, P.D.. F.,Urbach, and R.E. Davies (1979) Enhancement of experimental photocarcinogenesis by topical retinoic acid. Cancer Letters. 7 85-90 85

(2) Hickman, G.T., and J.T. Novak. (1989), Relationship between subsurface biodegradation rates and microbial density. Environmental Science and Technology 23(5): 525-5

(3) Brockmann H.E., FJ.deSerres, T. Ong, D.M.,DeMartin, AJ.,Katz, JF.,Griffiths, and R.S.,Stafford. (1984) Mutation tests in Neurospore crassa: A report of the US Environmental Protection Agency Gene-Tox Program: Mutat. Res., 133, 87-134

(4) Andrews,L.S., J.J.Clary, J.B.Terrill,and .F.Bolte. (1987).

Subchronic inhalation toxicity of methanol. J Toxicol. Environ. Health 20:117-124.

(5) Apaja, M, (1980) Evaluation of toxicity and carcinogenicity of malonaldehyde Acta Univ. Oul D55. Anat. Pathol Microbiol.

(6) Atkinson R.A., (1989) J Phys. hem. Ref. Data Monograph 1 p245

(7) Atkinson, R.A., (1990) Atomspheric Envirnoment 24 A 1-41

(8) Barton, D.A., and J.J. McKeown. (1991. Field verification of predictive modeling of organic compound removal by biological wastewater treatment processes. Environmental Progress 10(2): 96-103.

(9) BASF AG (1961) unpublished data

(10) BASF AG (1975) unpublished data

(11) BASF AG (1980) unpublished data

(12) Bengtsson, B.E., L Renberg and M. Tarkea (1984), Molecular structure and aquatic toxicity - example with C1-C13 aliphatic alcohols, Chemosphere 13(5/6): 613-622.

(13) Bolon, B., D.C. Dorman, D. Janszen, K.T. Morgan, and F. Welsch. (1993). Phase-specific developmental toxicity in mice following maternal methanol inhalation. Fund. Appl. Toxicol.21:508-516.

(14) Bolon, B., F. Welsch, and K.T. Morgan. (1994). Methanol-induced neural tube defects in mice:Pathogenesis during neurulation. Teratology 49:497-517.

(15) Boubilik T et al., (1984) The vapor pressure of pure substances: selected values of the temperature dependence of the vapor pressure of some pure substances in the normal and low pressure region Vol 17, Amsterdam, Netherlands: Elsevier Sci. Publ

(16) Burbacher, T., D.,Shen, K.,Grant, L.,Sheppard, D.,Dumian, S.,Ellis andc N.,Liberato, (1999) Reproductive and offspring developmental effects following maternal inhalation exposure to methanol in non-human primates, Part 11: Developmental effects in infants exposed prenatally to methanol. Health Effects Institute Report 89 October 1999

(17) Burbacher, T., D.,Shen, K.,Grant, L.,Sheppard, D.,Dumian, S.,Ellis andc N.,Liberato, (1999) Reproductive and offspring developmental effects following maternal inhalation exposure to methanol in non-human primates, Part 1: Methanol disposition and reproductive toxicity in adult female , Health Effects Institute Report 89 October 1999

(18) Cameron, A.M., O.G. Nilsen, E. Haug, and K.B. Eik-Nes.

(1984). Circulating concentrations of testosterone, luteinizing hormone and follicle stimulating hormone in male rats after inhalation of methanol. Arch. Toxicol. Suppl. 7:441-443.

(19) Campbell, J.A., D.R. Howard, L.C. Backer, and J.W. Allen. (1991) Evidence that methanol inhalation does not induce chromosome damage in mice. Mutation Res. 260: 257-264.

(20) Chikin M.S., and K.S.,Shadurskii (1969) Farmakol Toksikol 32:90-94

(21) cited in Rowe. VK. and, S.B., McCollister,. (1982), Alcohols. In Pattv's Industrial Hygiene and Toxicology. Third Edition. Vol. 2C. (Clayton. C. D. and Clayton, F.E. eds.) John Wiley & Sons, New York. pp. 4528-4541.

(22) Connelly, LE and JM. Rogers (1997) Methanol Causes Posteriorization of Cervical Vertebrae in Mice Teratology 55:138-144

(23) Cooper J.R., and P., Felig , (1961) The biochemistry of methanol poisoning II Metabolic acidosis in the monkey, Toxicol. Appl. Pharmacol. 3:202-209

(24) Cooper,R.L.,; M.L., Mole, G.L., Rehnberg; J.M., Goldman; W.K., McElroy; J., Hein; T.E.,Stoker (1992),Effect of inhaled methanol on pituitary and testicular hormones in chamber acclimated and non-acclimated rats. Toxicology; 71:69-81

(25) Cornish H.H., and J. Adefuin , (1967), Potentiation of carbon terachloride toxicity by aliphatic alcohols, Arch Environ Health 14:447-449

(26) Cummings, A.M., (1993) Evaluation of the effects of methanol during early pregnancy in the rat. Toxicology 24;79 (3):205-14

(27) De-Carvalho, R.R., I.F., Delgado, C.A.,Souza, I., Chahoud and F.J.,Paumgartten , (1994), Embryotoxicity of methanol in well-nourished and malnourished rats, Brazilian J. Med. Biol. Res. 27, 2915-2923.

(28) Deichmann W.B. and E.G., Mergard, (1948), Comparative evaluation of methods employed to express the degree of toxicity of a compound J. Ind Hyg. Toxicol. 30:373-378

(29) Dorman, D.C., (1993). Acute Methanol Toxicity in Minipigs.. Fundam. AppL ToxicoL 20, 341-347.

(30) DuPont unpublished data

(31) Environ Corporation (1996) Hazard Assessment for humans and environmental health methanol,
Report for American Forest and Paper Association Inc

(32) Environ Corporation (1996) Hazard Assessment for humans and environmental health methanol, Report for American Forest and Paper Association Inc

(33) Ewell, W.S., J.W. Gorsuch, R.O. Kringle, K.A. Robillard, and R.C. Spiegel. (1986). Simultaneous evaluation of the acute effects of chemicals on seven aquatic species. Environmental Toxicology and Chemistry 5(9): 831-840.

(34) Flick, E.W., (1985) Industrial Solvent Handbook. 3rd ed Park Ridge, NJ, Noyes Publications 188

(35) Fu, S.S., T.M., Sakanashi , J.M.,Rogers , K.H.,Hong, C.L., Keen, (1996), Influence of dietary folic acid on the developmental toxicity of methanol and the frequency of chromosomal breakage in the CD-1 mouse Reprod Toxicol 10(6):455-63

(36) Hansch, C., Leo, A., Hoekman (1995) Exploring QSAR -, Hydrophobic, Electronic and Steric Constants, Washington, DC: American Chemical Society

(37) Hazardous Substance Database (HSDB) (1999), National Library of Medicine, National Institutes of Health

(38) Howard, P.H. (1990). Handbook of environmental fate and exposure data for organic chemicals. Chelsea, Michigan: Lewis Publishers.

(39) Howard, P.H. (1991). Handbook of Environmental Degradation Rates. Chelsea, Michigan: Lewis Publishers.

(40) Howard, P.H., (ed) (1990) Handbook of environmental fate and exposure data for organic chemicals, Vol II Solvents, Lewis Publishers Inc. Michigan

(41) Hwang H.M., R.E. HHHodson ans R\D.L. Lewis (1989) Assessing interaction of organic compounds during biodegradation of complex waste mixtures by naturally occurring bacterial assemblages, Environ. Toxicol. Chem; 8:209-214

(42) Infurna, R., and B. Weiss. (1986), Neonatal behavioral toxicity in rats following prenatal exposure to methanol.Teratology 33:259-265.

(43) Jacobs, G.A. (1990), Acute Data, J. Amer. Coll. Toxicol. 1 :56-58

(44) Keller, A., (1993) Acute toxicity of several pesticides, organic compounds, and a wastewater effluent to the freshwater mussel, Anodonta imbecillis, Ceriodaphnia dubia and Pimephales promeias, Bull. Envirin. Contam. Toxicol 51:696-702

(45) Kimura ET, D.M., Ebert, and P.W., Dodge., (1971) Acute toxicity and limits of solvent residue for sixteen organic solvents, Toxicol. Appl. Pharmacol. 19:699-704

(46) Kloepffer, W., Kohl, E.G., (1991) Ecotoxicol. Environ.Safety

(47) Kuhn, R., M. Pattard, K. Pemak, and A. Winter. (1989). Result of harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to Daphina magna Water Research 23(4): 495-499.

(48) Lasne-C; GU-Z.W; Venegas-W; Chouroulinkov-I (1984), The in vitro micronucleus assay for detection of cytogenetic effects induced by mutagen-carcinogens: comparison with the in vitro sister-chromatid exchange assay. Mutat-Res; 130:273-82

(49) Lee-E; A.N., Brady; M.J.,Brabec, T.,Fabel, (1991) Effects of methanol vapors on testosterone production and testis morphology in rats. Toxicol-Ind-Health; 7:261-275.

(50) Lewis P.A.,and Weber C.I.,(1985) A study of the relabilty of Daphnia acute toxicity tests. In Cardwell R.D. Purdy R., Bahner R.C., (ed) Aquatic toxicology and hazard assement:7th symposium ASTM STP 854 ASTM Philadelphia PA pp73-86

(51) Lyman, W.J. et al., (1982) Handbook of chemical mproperties estimation methods, McGraw-Hill, New York, Chapter 16.

(52) Lyman, W.J. et al., (1982) Handbook of Chemical Properties Estimation Methods, McGraw-Hill, New York 7-4

(53) McGregor, D.B. R., Martin, C.G., Black and W.J.,. Caspary (1985): Optimisation of a metabolic activation system for use in mouse lymphoma L5178Ytx+tx- mutation system. Environ. Mutagen. 7 (suppl. 3), 10

(54) Munch J.C., (1972), Aliphatic alchols and alkyl esters:narcotic and lethal potencies to tadpole and to rabbits, Ind. Med. Surg. 41:31-33

(55) Nelson, B.K., W.S. Brightwell, D.R. MacKenzie, A. Khan, J.R. Burg, W.W. Weigel, and P.T. Goad. (1985). Teratological Assessment of methanol and ethanol at high inhalation levels in rats. Fundamental and Applied Toxicol 5: 727-736.

(56) New Energy Development Organization (NEDO). (1986). Toxicological research of methanol as a fuel for power station. Tokyo, Japan. September.

(57) New Energy Development Organization (NEDO). (1986). Toxicological research of methanol as a fuel for power station. Tokyo, Japan. September

(58) New Energy Development Organization (NEDO). (1986). Toxicological research of methanol as a fuel for power station. Tokyo, Japan. September.

(59) O'Loughlin, K. S. LaValley, J. Mirsalis and J. McGregor,(1992),

Erthrocyte Micronucleus assay of methanol in normal and folate deficient swiss mice. : Environ. Mol. Mutagen. Suppl. 20, 47

(61) Pirnie, M., (1999) Evaluation of the fate and transport of methanol in the environment. American Methanol Institute Report

(62) Poirier, S.H., M.L. Knuth, C.A. Anderson-Bucho, L.T. Brooke, A.R. Lima, and P.J. Shubat. (1986), Comparative toxicity of methanol and N,N,-dimethylformamide to freshwater fish and invertebrates. Bull. Environ.Contam. Toxicol. 37: 615-621.

(63) Price, K.S., G.T. Waggy, and R.A. Conway. (1974). Brine shrimp bioassay and seawater BOD of petrochemicals. Journal of the Water Pollution Control Federation 46(l): 63-77.

(64) Pugh, L.B.; B.J.,. Ian and C.E., Carey (1996), Bioremediation of contaminated soils and groundwater at a former solvent storage site. Biotechnol. Ind. Waste Treat. Biorem. page 195 - 211.

(65) Rogers,J.M.; M.L., Mole; (1997) Critical periods of sensitivity to the developmental toxicity of inhaled methanol in the CD-1 mouse, Teratology 55:364-371.

(66) Rogers,J.M.; M.L., Mole; N., Chernoff; B.D.,Barbee; C.L., Turner; T.R., Logsdon; R.J.,Kavlock, (1993), The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses. Teratology 47(3):175-88

(67) RTECS (1994)

(68) Ryan, B.M. , N.S., Hartoum, E.J., Mallett and J.K., Yermakoff, (1994) A pilot developmental toxicity study of methanol in folate-deficent Long-Evans rats, Teratology 49, 399

(69) Sakanashi, T. M , J.M. ,Rogers, S.S., Fu, L.E., Connelly and C.L., Keen, (1996) Influence of Maternal Folate Status on the Developmental Toxicity of and in the CD-1 Mouse Teratology 54:198-206

(70) Sayers. R.R.. W.P.,Yant, H.H., Schrenk, J., Chornyak., S. J., Pearce, F.A. Patty, and J.G., Linn,. (1942). Methanol poisoning. I. Exposure of dogs to 450-500 ppm methanol vapors in air. Report of Investigations RI 3617 U.S. Dept. of Interior, Bureau of Mines. pp. 1-10,

(71) Sayers. R.R.. W.P.,Yant, H.H., Schrenk, J., Chornyak., S. J., Pearce, F.A. Patty, and J.G., Linn,.. (1944), Methanol poisoning. II. Exposure of dogs for brief periods eight times daily to high concentrations of methanol vapor in air. J. Industr. Hyg. Toxicol. 26:255-259,

(72) Schultz, T.W., T.S., Arnold, T.S., Wilke, and M.R.,

Moulton, (1990) Relationship of quantitative structure-activity for normal aliphatic alcohols, Ecotoxicology and Environmental Safety, 19: 243-253.

(73) Scott, J.B.,F.A., Smith, and R.H., Garman, (1979) Exposure to mice to CH2CL2 and CH3OH and in combination, Toxicol. Appl. Pharmacol. 48:105A

(74) Shimizu H., Y., Suzuki, N., Takemura, S., Goto, and Matsushita (1985), The results of microbial mutation test for forty-three industrial chemicals : Jpn. J. Ind. Health, 27, 400-419.

(75) Skirko et at (1976) cited in Rowe. VK. and, S.B., McCollister,. (1982), Alcohols. In Pattv's Industrial Hygiene and Toxicology. Third Edition. Vol. 2C. (Clayton. C. D. and Clayton, F.E. eds.) John Wiley & Sons, New York. pp. 4528-4541.

(76) Smith E.N., and R.T., Taylor, (1982) Acute toxicity of methanol in the folate defiecent acatasemic mouse, Toxicology 25:271-287

(77) Smyth H.F, J., Seaton and L., Fischer , (1941), The single dose toxicity of some glycol and derivatives, J. Ind. Hyg. Toxicol 23:259-268

(78) Smyth unpublished data

(79) Stanton, M.E., K.M. Crofton, L.E. Gray, C.J. Gordon, W.K. Boyes, M.L. Mole, D.B. Peele, and P.J. Bushnell. (1995), Assessment of offspring development and behavior following gestational exposure to inhaled methanol in the rat. Fund Appi. Toxicol. 28: 100-110.

(80) Stern S., C., Cox, R., Preston , A., Sharma A., G.B.,Ingles, M., Balys, K.R., Reuhl, and B.,Weiss, (1997). Perinatal methanol exposure in the rat. II Behavioral effects in neonates and adults. Fund Appi. Toxicol. 36: 163-176.

(81) Stratton,G.W., T.M.,Smith, (1988), Interaction of organic solvents with the green alga Chlorella pyrenoidosa, Bull. Environ. Contam. Toxicol. 40(5), 736-742

(82) Sufllta, J.M., and M.R. Mormlle. (1993), Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface. Environmental Science and Technology 27: 976-978.

(83) Swain, H.M. and Somerville< H.J. (1978) Microbial metabolism of methanol in a model activated sludge system, J. Appl. Bacterl. 45:147-151

(84) The Merck Index (1983) 10ed Rathway, N.J. Merck Co Inc 853

(85) Union Carbide data

(86) USEPA 1986.

(87) Veith, G.D., D.J. Call and L.T. Brooke (1983), Structure-toxicity relationship for the fathead minnow, Pimephales promelas: narcotic industrial chemicals. Can.J.Fish Aquat.Sci., 40 743-748.

(88) Von Burg R J (1994), Toxicology update Appl Toxicol; 14(4):3 09-13

(89) Von Burg R J (1994), Toxicology update Appl Toxicol; 14(4):3 09-13 .

(90) Weil C. (1972) Statistics vs safety factors and scientific judgment in the evaluation of safety for man,Toxicol. Appl. Pharmacol. 21:454-463

(91) Welch H., and G.G., Slocum, (1943) J. Lab. Clin. Med. 28:1440

(92) White, L.R., A.B.L. Marthinsen, R.J. Richards, K.B. Eik-Nes, and O.G. Nusen. (1983). Biochemical and cytological studies of rat lung after inhalation of methanol vapor. Toxicol.Lett. 17:1-5.

(93) World Health Organization (WHO) (1997) Methanol, Environmental Health Criteria; 196; 180 p

(94) Witte, R., (1931) Disseration, Wurzburg

(95) World Health Organization (WHO) (1997) Methanol, Environmental Health Criteria; 196; 180 p

(96) Yaws C.L. (1990) Chemical Eng. p115-118 July

SUPPLEMENT REFERENCES (Introduction an Discussion)

Andrews,L.S.,J.J.Clary, J.B.Terrill,and .F.Bolte. (1987). Subchronic inhalation toxicity of methanol. J *Toxicol. Environ. Health* 20:117-124.

Batterman, SA., and A. Franzblau (1997), Time-resolved cutaneous absorption and permeation rates of methanol in human volunteers, *Int Arch Occup Environ Health* 70: 341-351

Burbacher, T., D.,Shen, K.,Grant, L.,Sheppard, D.,Dumian, S.,Ellis andc N.,Liberato, (1999) Reproductive and offspring developmental effects following maternal inhalation exposure to methanol in non-human primates, Part 1: Methanol disposition and reproductive toxicity in adult female , *Health Effects Institute Report 89* October 1999

Horton, V.L., M.A. Higuchi, and D.E. Rickert. (1992) Physiologically baseed pharmacokinetic model for methanol in rats, monkeys and humans, *Toxicol. Appl. Pharmacol.* 117:26-35.

Kavet, R., and K.M. Knauss. (1990). The toxicology of inhaled methanol vapors. *Critical Reviews in Toxicology* 21(l): 22-50.

Lee-E.W., T.S. Terzo, J.B. D'Arcy, K.B. Gross, and R.M. Schreck, (1992) Lack of blood formate accumulation in humans following exposure to methanol vapor at the current permissible exposure limit of 200 ppm. *Am. Ind. Hyg. Assoc.* 53:99-104.

Leon A.S., D.B. Hunninghake, C. Bell, D.K. Rassin and T.R. Tephly, (1989) Safety of long-term large doses of aspartame. *Arch. Intern. Med*. 49:2318-2324.

Martinasevic, M. K., M.D., Green, J.,Baron,, and T.J.,Tephly, (1996) Folate and 10-Formyltetrahydrofolate Dehydrogenase in Human and Rat Retina: Relation to Methanol Toxicity.. *Toxicol. Appl. Pharmacol.* 141: 373-381.

New Energy Development Organization (NEDO). (1986). Toxicological research of methanol as a fuel for power station. Tokyo, Japan. September.

Perkin RA., K W. Ward, and G M. Pollack (1995), A Pharmacokinatic Model of Inhaled Methanol in Humans and Comparison to Methanol Disposition in Mice and Rats, *Environ. Health Perspect* 103: 726-733

Raabe, OG., A., Bayati., P.D., Schulec, , F., Gielow, D.,Uyeminami, and N.,Shimasaki, (1992) Dermal absorption of methanol and methanol/gasoline mixture, Final report for Research Division California Air Resources Board Contract No. A933-186.

Roe, 0. (1982), Species differences in methanol poisoning. *CRC Critical Reviews in Toxicology,* 10: 275-286.

Tephly, T.R. (1991). The toxicity of methanol. *Life Sciences* 48: 1031-1041.

Tephly, TR., and K.E.,McMartin, (1984), Methanol metabolism and toxicity, in aspartame: *Physiology and Biochemistry*, Stegink. L.D. and Filer, L.J., Jr., eds., Marcel Dekker, New York, pp. 111-140.

World Health Organization (WHO) (1997) Methanol, *Environmental Health Criteria; 196; 180* p